Metabolically Assessed Fibre Recruitment

Beltman, J.G.M.

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Marijke Beltman

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Metabolically Assessed Fibre Recruitment

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promotoren: prof.dr. A. de Haan
prof.dr. A.J. Sargeant

Metabolically Assessed Fibre Recruitment

Johanna Gerharda Maria Beltman

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CONTENTS

Chapter 1	Introduction	9
Chapter 2	Effect of antagonist muscle fatigue on knee extension torque	17
Chapter 3	Changes in PCr/Cr ratio in single characterized muscle fibre fragments after only a few maximal voluntary contractions in humans	35
Chapter 4	Metabolically assessed muscle fibre recruitment in brief isometric contractions at different intensities	49
Chapter 5	Metabolic cost of lengthening, isometric and shortening contractions in maximally activated rat skeletal muscle	63
Chapter 6	Lower voluntary activation of human quadriceps muscle during lengthening contractions: no evidence for selective recruitment of type II fibres	79
Chapter 7	Summarising discussion/samenvatting en discussie	99
References		117
Dankwoord		133
List of Publications		137

CHAPTER 1

Introduction

INTRODUCTION

The motor unit

The functional unit for muscular activity is a motor unit. It consists of a motoneuron and all the muscle fibres innervated by this motoneuron. When an action potential is propagated in a single motoneuron, all fibres in that motor unit are stimulated and contract. On the basis of physiological properties motor units can be divided into slow, fatigue resistant (type S) and fast (type F) contracting motor units (Burke et al., 1973). In the type F motor units two groups can be distinguished, the fast-fatigue resistant (FR) and the fast-fatigable (type FF) units (Burke et al., 1973).

The activation pattern of the innervating motoneuron is an important factor determining the type of the muscle fibres in a motor unit. Therefore, it is generally assumed that all muscle fibres of a given motor unit have the same contractile and metabolic properties that is, that the fibres are of the same type (Saltin and Gollnick, 1983). Human skeletal muscle contains two major fibre types, type I and type II. The latter can further be subdivided into two subgroups IIA and IIX (Ennion et al., 1995; Sant'Ana Pereira et al., 1995a). Type I fibres are known to be slow-oxidative, type IIA fibres fast-oxidative-glycolytic and type IIX are considered to be the fast-glycolytic fibres. However, a continuum exists such that fibres can also express combinations of myosin isoforms, for example type IIAX fibres are a hybrid type in which fibres co-express type IIA and IIX myosin isoforms.

Force regulation

Human skeletal muscle is designed to execute both fine and gross movements. By varying the degree of force generated, it is possible to engage in a variety of motor tasks. Two main mechanisms are available to control the amount of force needed for the activity. The first mechanism is based on the size of the motor units and is known as the *size principle* of Henneman et al. (1965). According to this principle the smaller (low force, fatigue resistant) units are recruited first. With increasing force requirement the larger (high force, fast fatigable) units are activated. This way the force is increased in a stepwise fashion. As well as varying the number and size of motor units recruited, muscle force can also be regulated by changing the firing rate of each activated motor unit. This second mechanism to control force is known as *rate coding* (e.g. Person and Kudina, 1972). This way of modulating force provides a sensitive control for human activity. To vary the amount of torque these two mechanisms are used simultaneously. Yet, there appears to be some variation in the extent in which they are used between muscles. For example, small muscles, such as the adductor pollicis, seem to rely heavily on rate coding while in larger muscles recruitment is the main mechanism to regulate force (Kukulka and Clamann, 1981). Moreover, there is some lack of knowledge on the extent to which both recruitment and the firing rate are used with respect to intensity of exercise. Though studies in human whole body exercise, such as cycling, have shown that there is an hierarchical recruitment of motor units with type I fibres recruited first and, at higher force levels, type IIA and IIAX fibres (Gollnick et al., 1973a; Gollnick et al., 1974a; Gollnick et al., 1974b; Vøllestad et al., 1984), there is also some evidence that suggests that both type I and IIA fibres are already recruited at low intensities of exercise (Ivy et al., 1987; Vøllestad et al., 1984). This suggests that rate coding of type II fibres may also play an important role at submaximal activation levels (Sargeant and Jones, 1995).

Though the order of recruitment seems to be rather fixed by the size principle in isometric contractions, there is some debate on the recruitment order during lengthening contractions. During these type of contractions voluntary activation level has been shown to be reduced (Babault et al., 2001). This reduced neural drive has been suggested to be caused by either a lower activation level of all recruited fibre populations and/or by a recruitment of type II fibres and de-recruitment of type I fibres (Enoka, 1996).

Studying recruitment patterns

A frequently used method to study muscle fibre recruitment patterns is the glycogen depletion method. The degree of glycogen depletion in different fibres is used as an indication of fibre activation (for review see Saltin and Gollnick, 1983). Glycogen, stored in skeletal muscle fibres, is known to become depleted when the fibres are used in prolonged exercise. Using the Periodic Acid Schiff reaction (PAS) the glycogen content can be measured in separate muscle fibres. A decrease in the PAS staining intensity in a fibre compared to rest is used as an indication that the fibre had been active. Using this method it has been shown that, during cycling exercise at relatively low intensities, type I fibres are recruited first and, with increasing duration or intensity of exercise, type II fibres are progressively recruited (e.g. Gollnick et al., 1973a; Gollnick et al., 1974b; Vøllestad et al., 1984; Vøllestad and Blom, 1985). One of the major limitations of this method is that an exercise of relatively long duration (~>10 min) is necessary to detect a decline in the PAS staining intensities during which there may be sequential recruitment of successive fibre type populations consequent upon metabolic depletion and/or fatigue of the fibres recruited initially. Therefore, limited conclusions can be drawn with respect to the order of recruitment from the start of exercise as both exercise duration and intensity may determine recruitment of fibre populations.

Another method to investigate recruitment patterns of motor units is to measure electromyographic (EMG) activity of single motor units using a needle or fine wire electrode inserted into the muscle (e.g. Carpentier et al., 2001; De Luca et al., 1982; Desmedt and Godaux, 1977a; Feiereisen et al., 1997; Freund et al., 1974; Grimby and Hannerz, 1977; Milner-Brown et al., 1973; Person and Kudina, 1972). This method was first introduced by Adrian and Bronk in 1929 and aims to detect motor unit action potentials of single fibres. The technique as developed and applied by a number of laboratories is very powerful allowing several parameters of the potentials to be measured, such as frequency, amplitude and recruitment threshold, which yields valuable insights into recruitment patterns of different motor unit types during single contractions. Most is known about motor unit behaviour during submaximal isometric contractions in small muscles. During high intensity contractions it is more difficult to recognise firing patterns of individual motor units due to simultaneous firing of many motor units in the area of the electrode (McComas, 1996). Measurements during dynamic contractions are also difficult as the movement of the muscle belly during the contraction can move the electrodes (McComas, 1996). Attempts have been made to assess recruitment patterns during high intensity isometric contractions and dynamic contractions (Linnamo et al., 2003; Moritani and Muro, 1987) using intramuscular EMG. However, this method can only discriminate motor unit pools and does not allow identification of single motor units.

Each of these methods has clearly its own potential and possibilities. However, with the available methods it is not possible to assess recruitment patterns of different fibre types (i.e. type I, IIA and IIAX) in (near) maximal isometric, and various modes of dynamic exercise, without interference of fatigue. In the present thesis a new technique is developed and applied that uses a metabolic parameter to study the recruitment of single fibres after very brief exercise at different intensities and modes of exercise.

PCr/Cr ratio as a marker for fibre activation

Muscle performance is dependent on available energy in the form of ATP. Skeletal muscle contains only a small amount of ATP, which would be used up within a few seconds during maximal activation. Therefore, ATP has to be replenished very rapidly. Phosphocreatine (PCr) is the main energy source during the first few seconds of exercise. As ATP is used for contractions it is rapidly resynthesised from PCr, which is broken down to creatine (Cr) by donating a phosphate group to ADP, a reaction that is catalysed by the enzyme creatine kinase:

$$PCr + ADP + H^{+} \xleftrightarrow{\text{creatine kinase}} Cr + ATP$$

In a resting muscle, the PCr store is approximately 3 to 4 times greater than the muscle ATP store, so initially there is sufficient PCr available to immediately resynthesise ATP (Hultman et al., 1990). While other ATP regenerating mechanisms are also activated once the exercise has started they do not provide ATP instantaneously. Therefore, the main role of PCr during the first seconds of exercise is that of a temporal energy buffer.

Over the past years, a method has been developed in our laboratory, which enables us to measure metabolites in human single characterized muscle fibre fragments (Karatzaferi et al., 1999; Sant'Ana Pereira et al., 1995b)(Figure 1). Needle biopsies of the vastus lateralis muscle are obtained at rest and immediately after exercise. These biopsies are immediately frozen in liquid nitrogen and freeze dried. Single muscle fibre fragments are dissected from the freeze-dried samples and classified as type I, IIA or IIAX, using mATPase stability-based histochemistry. Subsequently, individual fragments of characterized fibres can be analysed for adenine nucleotides (ATP, IMP) and creatine compounds (PCr and Cr). Using this technique it has been shown that PCr levels decrease very rapidly during maximal exercise: to near zero after 25 s of maximal cycling (Karatzaferi et al., 2001a; Sant'Ana Pereira et al., 1996). Even after 10 s of maximal cycling there was a great decline of the PCr levels to ~50% of resting levels (Karatzaferi et al., 2001b). Thus, this newly developed method was shown to be very useful for studying metabolite changes in single fibres during and after short-term fatiguing whole body exercise.

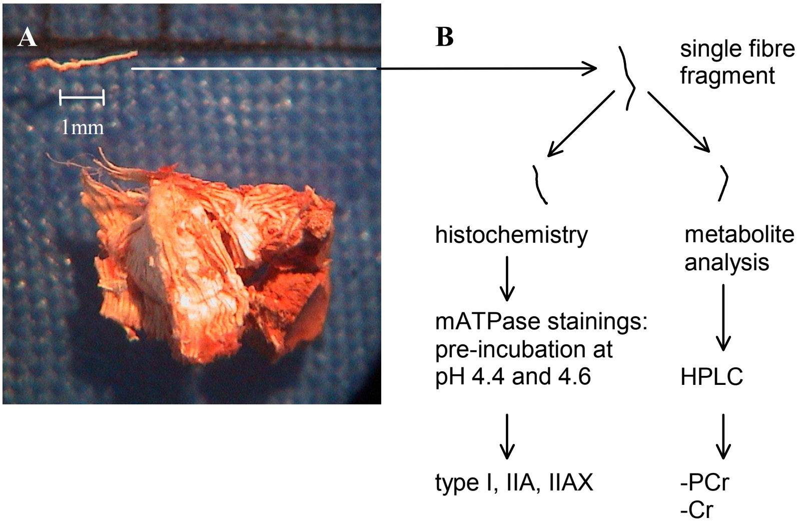

Figure 1. Schematic representation of the analysis of muscle samples. A) Part of a freeze-dried muscle sample, along with a dissected fibre fragment of ~2 mm. B) Under controlled ambient conditions (20-25°C and <35% RH) single fibre fragments were dissected and each fragment was cut into two pieces. The first fragment was prepared for histochemistry; mATP-ase stainings with a pre-incubation at pH 4.4 and 4.6 allowed the fibre fragments to be characterized. The intensity of the staining with pre-incubation at pH 4.4 was used to classify the fibres in type I and II, while the intensity of the staining with pre-incubation at pH 4.6 was used to divide the type II fibres in type IIA and IIAX. The remaining fragments of the characterized fibres were used for metabolite analysis. Of each fibre type a maximum of ~20 fragments was analysed for phosphocreatine (PCr) and creatine (Cr) by using reverse-phase high-performance liquid chromatography (HPLC) with ultra-violet photometric detection.

The present thesis aims to apply this method to investigate recruitment patterns of different fibre types during brief exercise of the knee extensors on a dynamometer (Figure 2). For this purpose the ratio of PCr to Cr (PCr/Cr) was used as an indication of activation of human muscle fibres. As the creatine-kinase reaction is in near equilibrium, the PCr/Cr ratio is directly related to the ATP/ADP ratio, which is a measure for the energetic state of a muscle fibre (Conjard et al., 1998). Therefore, it can be expected that the PCr/Cr ratio is a sensitive marker for muscle fibre activation. The advantage of this metabolic approach compared with other available methods is that it would allow studying recruitment patterns of different fibre types (type I, IIA and IIAX) during the initial period of exercise. The second advantage would be that both high intensity isometric as well as dynamic contractions could be studied.

Figure 2. Subject on the custom-built dynamometer that was used for isometric and dynamic knee extension contractions in Chapter 3, 4 and 6.

AIM OF THIS THESIS

Knowledge of the mechanisms regulating the force production of muscles is essential for understanding the control of normal and pathological movements. In addition, an understanding of recruitment patterns is important in the design of both neuromuscular rehabilitation of patient groups and athletic training. Recruitment of motor units or muscle fibres has been a subject of interest in many studies. It is clear, however, that there is still a lack of knowledge regarding recruitment patterns of different fibre types during the initial period of exercise. In addition, there is no consensus on the recruitment patterns during lengthening contractions. Therefore, the main aim of this thesis is to study recruitment of different fibre types (type I, IIA and IIAX) at several intensities and modes of brief exercise of the knee extensors using the PCr/Cr ratio in single characterized fibre fragments as a measure of fibre activation.

OUTLINE OF THIS THESIS

Several specific research questions are addressed in the present thesis:

1. *Can we use the human quadriceps muscle as a model for our studies? What is the possible influence of the co-contracting antagonist hamstring muscle on net extensor torque output at different knee angles?* Chapter 2 describes an experiment, which investigated the influence of antagonist hamstring fatigue on knee extensor torque.
2. *Can the PCr/Cr ratio be used to study activation of single muscle fibres in humans?* In Chapter 3 a protocol is developed to detect fibre activation using the PCr/Cr ratio of single characterized fibre fragments after very brief exercise of only a few seconds. In this experiment we measured the PCr/Cr ratio in different fibre types after a series of 4, 7 and 10 isometric contractions to identify the exercise duration necessary for the detection of activated fibres.
3. *What is the recruitment pattern of human muscle fibre types at different intensities of brief isometric exercise by measurement of the PCr/Cr ratio and what is the contribution of rate coding?* Chapter 4 describes this experiment in which the PCr/Cr ratio was determined in type I, IIA and IIAX fibres at rest and after series of 7 isometric contractions performed at 40, 70 and 100% of maximal voluntary contraction torque.
4. *Is there a difference in the metabolic cost of maximally activated skeletal muscles during short-term lengthening, isometric and shortening contractions at velocities that are comparable with movements in vivo?* In Chapter 5 this experiment is described, which studied electrically stimulated rat medial gastrocnemius muscles. Series of 10 maximal lengthening, isometric and shortening contractions were performed and the high-energy phosphate consumption of these three modes of contraction was determined and related to force production.
5. *Is there a difference in voluntary activation level during lengthening, isometric and shortening contractions? If so, is there evidence that this is caused by a preferential recruitment of human type II fibres?* In Chapter 6 the voluntary activation level during lengthening, isometric and shortening contractions was determined in humans using a superimposed stimulation technique. In addition, the PCr/Cr ratio was determined in type I, IIA and IIAX fibres after these three modes of contraction to investigate the recruitment pattern of these fibre types.

Finally, in Chapter 7 the main results are summarised and discussed.

Chapter 2

Effect of antagonist muscle fatigue on knee extension torque

ABSTRACT

The effect of hamstring fatigue on knee extension torque was examined at different knee angles for seven male subjects. Before and after a dynamic flexion fatigue protocol (180°·s^{-1}, until dynamic torque had declined by 50%), maximal voluntary contraction (MVC) extension torque was measured at four knee flexion angles (90°, 70°, 50° and 30°). Maximal torque generating capacity (MTGC) and voluntary activation (VA) of the quadriceps muscle were determined using electrical stimulation. Average rectified EMG (EMG_{ar}) of the biceps femoris was determined. Mean dynamic flexion torque declined by 48 ± 11%. Extensor MVC torque, MTGC, VA and EMG_{ar} at the four knee angles were unaffected by the hamstring fatigue protocol. Only at 50° knee angle was VA significantly lower, 15.7%, after fatigue ($P<0.05$). In addition, EMG_{ar} before fatigue was not significantly influenced by knee angle. It was concluded that a fatigued hamstring muscle did not increase the MVC extension torque and knee angle did not change coactivation. Three possible mechanisms may explain the results: a potential difference in recruited fibre populations in antagonist activity compared with the fibres which were fatigued in the protocol, a smaller loss in isometric torque generating capacity of the hamstring muscle than was expected from the dynamic measurements and/or a reduction in VA.

INTRODUCTION

The influence of antagonist activity on knee extension torque is important in dynamometry because coactivation affects the net torque generated around the joint. It has been proposed that this antagonist activity may have functional significance in stabilising the joint and/or protecting the joint at extreme knee angles (Baratta et al., 1988; Osternig et al., 1995; Solomonow et al., 1987).

A number of reports have shown significant EMG activity levels of the antagonist hamstring muscles around the knee joint in static and dynamic knee extensions (Aagaard et al., 2000a; Baratta et al., 1988; Eloranta, 1989; Grabiner et al., 1992; Hagood et al., 1990; Kellis, 1998; Osternig et al., 1984), indicating that substantial antagonist torque is produced in those contractions. At extended knee joint angles, quadriceps muscle contraction results in an anterior translation and internal rotation of the tibia, which can impose a high degree of stress on the anterior cruciate ligament (ACL) (Beynnon et al., 1992; Hirokawa et al., 1992; Kaufman et al., 1991; More et al., 1993; Nisell et al., 1989; Renstrom et al., 1986). It is suggested that mechanoreceptors in the ACL are excitated and that, by means of a ligament-muscle reflex loop, the hamstring muscle is activated to maintain joint stability (Solomonow et al., 1987). Cocontraction of the hamstring muscle at these angles decreases the anteriorly directed shear of the tibia (Draganich et al., 1989; Draganich and Vahey, 1990; Kaufman et al., 1991; More et al., 1993; Renstrom et al., 1986) and thus reduces the strain on the ACL (Draganich and Vahey, 1990; Kaufman et al., 1991; More et al., 1993; Yasuda and Sasaki, 1987). As the anterior displacement occurs mainly in the range of 15-45° knee flexion (where 0° is full extension) (Hirokawa et al., 1992), it might be expected that antagonist activity would vary with knee angle with the highest antagonist activity toward full extension.

While several studies (Aagaard et al., 2000a; Baratta et al., 1988; Hagood et al., 1990; Kellis and Baltzopoulos, 1996a) have indeed shown that, under dynamic conditions, the antagonist EMG activity varies with knee angle, Osternig et al. (1984) reported no difference in EMG activity between the first 100° and the last 25° of the knee extension motion (ROM ~115°). Under isometric conditions, Eloranta (1989) observed that antagonist activation increased toward the end of the knee extension, but Grabiner et al. (1992) reported no significant differences in hamstring activation levels at 15° and 85° of knee flexion.

The aim of this study was to investigate the influence of antagonist hamstring fatigue on net extension torque at different knee angles. As data on antagonist activity during isometric contractions is limited, this study focused on this type of contractions. We hypothesised that reducing the torque generating capacity of the antagonist hamstring muscles, by employing a fatiguing bout of flexion contractions, would result in an increase in net extension torque. In addition, we have measured EMG activity of the hamstring muscle to investigate the antagonist activity at different knee angles. We hypothesised that we would see higher antagonist EMG activity at smaller knee flexion angles.

METHODS

Subjects

Seven healthy, recreationally active, male subjects gave their written informed consent and volunteered to participate in the present study. Their mean ± SD age, height and body mass were 27 ± 8 years, 179 ± 5 cm and 76 ± 12 kg, respectively. The study was approved by the ethics committee of the Manchester Metropolitan University and was in accordance with the Declaration of Helsinki (2000).

Study design

Subjects performed maximal isometric knee extension contractions (MVC) on a dynamometer at different knee angles (90°, 70°, 50° and 30° knee flexion, 0° = full extension). A concentric fatigue protocol for the hamstring muscle was then performed until dynamic torque had declined by ~50%. After the last dynamic flexion contraction, an extension MVC was performed. Superimposed electrical stimulation was elicited on all extension MVCs and EMG activity of the biceps femoris was measured. Prior to the experiments the subjects came to the laboratory for two familiarisation sessions. In those sessions the subjects practised performing MVCs at different knee angles and they were familiarised with electrical stimulation. In addition, the number of contractions for the fatigue protocol was determined for each subject.

Electrical stimulation

To determine the activation level of the quadriceps muscle the superimposed stimulation technique has been used. Electrical stimulation was applied on the quadriceps muscle during each extension MVC and twice on the relaxed muscle (5 and 10 seconds after the voluntary contraction). A constant current stimulator (Model DS7, Digitimer, Welwyn Garden City, UK) was used with self-adhesive surface electrodes placed on the anterior of the thigh. One electrode was placed on the most prominent part of the m. vastus medialis; the second electrode was placed on the upper part of the thigh, as high as possible. Four square wave pulses of 50 μs were delivered to the muscle at a frequency of 100 Hz, using maximal current. This current, using a single pulse of 50 μs, was determined by raising the current until torque did not increase further. The first habituation session was used to determine the maximal current for each subject. The same current was used in the experimental session after checking whether it was still maximal current. If necessary, the current was adjusted. The mean current used was 486 ± 75 mA.

Isometric and isokinetic exercise

An isokinetic dynamometer (Lido Active, Loredon Biomedical, Davis, CA, USA) was used for isometric and isokinetic exercise. The subject was positioned on the seat of the dynamometer with a hip angle of 90° (0° = full extension). The axis of rotation of the dynamometer was aligned with the lateral femoral epicondyle. Seat belts at the lap and shoulder were used to stabilise the subject during force exertion. To keep the thigh in place, a padded roll pressed the thigh onto the seat. The lower leg was secured to the dynamometer arm with an ankle strap. To protect the ligaments of the knee joint from potential shear forces during extension contractions with fatigued knee flexors, a second pad was placed as high as possible at the proximal end of the tibia (~15 cm distal to the lateral femur condyle). This pad was attached at the same position during all conditions. A pad preventing all anterior displacement would presumably decrease the EMG activity of the antagonist muscle and increase the net extension torque. However, the pad used in this experiment could only prevent excessive shear forces expected as a result of the fatigued hamstring muscles. Therefore, we assume that this pad did not restrict the anterior displacement as would occur with a fresh hamstring muscle. Moreover, the pad did not prevent the internal rotation of the tibia and thus there could still be a high degree of stress on the anterior cruciate ligament.

Torque was calculated from the digitised (100 Hz) signal. All recorded torque values were corrected for the effect of gravity. After each dynamic fatigue protocol the dynamometer had to be switched from isokinetic to isometric mode (for extension MVC), which took about 30 seconds. To prevent recovery of the hamstring muscle in this period, a cuff was inflated (to 210 mmHg) around the upper region of the thigh to occlude the circulation. Measurements in the first habituation session revealed that the 30 seconds inflation of the cuff had no influence on MVC extension torque.

EMG measurements

Bipolar Ag-AgCl surface electrodes were used for EMG recordings (centre-to-centre distance 2 cm) of the long head of the biceps femoris (BF). The skin at the electrode site was shaved and cleaned with alcohol wipes. The electrodes were placed half way on a line between ischial tuberosity and fibula head (Kellis and Baltzopoulos, 1996b). The ground electrode was positioned on the patella of the same leg. EMG signals were recorded by amplifiers (gain 20) with a signal measurement bandwidth of 5-270 Hz (PORTI-17/AS, Twente Medical Systems International, Twente, The Netherlands). The analogue signal was sampled and converted to digital form at a sampling frequency of 1000 Hz using a 22-bit AD-converter. The input noise level was less than 1.5 μV and the common mode rejection ratio was higher than 90 dB. The digital EMG signal was then corrected for offset, high pass filtered at 10 Hz (2^{nd} order Butterworth filter bi-directional) and full-wave rectified. A synchronisation pulse was applied to synchronise EMG and torque recordings.

Experimental set-up

Subjects visited the laboratory three times, twice for a familiarisation trial and once for an experimental session. Figure 1 gives a schematic overview of the procedures in the three sessions.

<u>Familiarisation sessions</u>

The first session was used to familiarise the subjects with the testing procedures and electrical stimulation. After a standardised warm-up on a cycle-ergometer for 5 minutes, subjects performed two maximal voluntary isometric extension contractions (MVC) at four knee angles (90°, 70°, 50° and 30° knee flexion) with superimposed stimulation. MVCs lasted ~3-4 seconds and a rest of 1½ to 2 minutes was allowed between all maximal efforts.

The effect of the occlusion cuff on maximal extension torque was also investigated in the first habituation session. As this cuff was placed over the stimulation electrodes, the cuff was released before the post-fatigue extension MVC to prevent pressure on the electrodes during stimulation. To investigate whether this cuff had a decreasing effect on MVC torque, the subjects performed an MVC (at 90° knee angle) before and after the cuff was inflated. It appeared that the inflated cuff had no significant effect (paired t-test, $P>0.05$) on MVC torque (mean ± SD: 276 ± 77 Nm and 276 ± 74 Nm before and after cuff inflation, respectively).

Familiarisation sessions	
Session 1 *Familiarise with testing procedures and electrical stimulation* ▪ 5 min warm-up on cycle ergometer ▪ extension MVCs (90, 70, 50 and 30°) ▪ superimposed electrical stimulation ▪ effect occlusion cuff on MVC	**Session 2** *Determine fatigue protocol* ▪ 5 min warm-up on cycle ergometer ▪ pre-fatigue flexion MVC (90°) ▪ dynamic flexion contractions until torque = 50% of first contraction ▪ post-fatigue flexion MVC (90°)

Experimental session (session 3)
▪ 5 min warm-up on cycle ergometer ▪ flexion MVCs (2x) at 90, 70, 50 and 30° (for EMG normalisation) 1. pre-fatigue extension MVCs with superimposed stimulation (2x) at 90, 70, 50 and 30° knee flexion (randomly) 2. fatigue protocol 3. post-fatigue extension MVC with superimposed stimulation at 1^{st} knee angle 4. fatigue protocol 5. post-fatigue extension MVC with superimposed stimulation at 2^{nd} knee angle ▪ 20 minutes active rest ▪ repeat step 1 to 5 (replace 1^{st} and 2^{nd} in step 3 and 5 by 3^{rd} and 4^{th})

Figure 1. Schematic overview of the protocols used in each session.

In the second habituation session, the fatigue protocol for each subject was determined. Before the fatiguing exercise, subjects performed a flexion MVC (90° knee angle) as a baseline measurement. Subjects then performed two series of maximal concentric flexion contractions (20°-90° knee flexion, $180°\cdot s^{-1}$) with passive extension, which was achieved by the experimenter lifting the leg during the extension phase. Torque was displayed online, and when dynamic torque had declined about 50%, this indicated the end of exercise. Before starting the second series of fatiguing contractions, a one-minute rest was provided. The second protocol was also terminated when the torque had declined by 50% of the initial contraction of the first series. Immediately after the last contraction, the occlusion cuff was inflated around the upper thigh. After about 30 seconds, the cuff was deflated and the subjects performed a flexion MVC (90° knee angle) as a post-fatigue measurement. The number of contractions performed and the decline in dynamic and isometric torque after these series determined the number of contractions of the experimental fatiguing protocol.

Experimental session

In the experimental visit, the subjects also performed a standardised warm-up on a cycle-ergometer for 5 minutes. Each subject performed two flexion MVCs at each knee angle (for EMG normalisation purposes), followed by two extension MVCs at each knee angle with superimposed stimulation. Knee angles (90°, 70°, 50° and 30° knee flexion) were randomised using a Latin square design. These contractions were followed by the first fatiguing bout of flexion contractions. Immediately after the last contraction, the occlusion cuff was inflated. Directly after deflating the cuff, the subjects performed an extension MVC at a certain knee angle. Next, the subject completed the second bout of fatiguing contractions, after which the cuff was inflated, deflated, and an extension MVC was performed at another knee angle. After this protocol, a 20-minute rest was allowed, during which the subjects could walk around and stretch their leg muscles. After the rest period, the two fatiguing protocols were repeated; however, the extension MVCs were performed at two different knee angles. The advantage of performing two fatiguing bouts consecutively was that, due to the cumulative effect of muscle fatigue, fewer contractions were needed in the second bout to reach the same degree of fatigue. The second advantage was that, with this set-up the duration of the session was kept acceptable.

At all contractions, isometric as well as concentric, subjects were verbally encouraged to perform their best. To prevent subjects from trying to reserve energy for the last of the fatiguing contractions, they were not told how many contractions they had to perform.

Data analysis

<u>Torque</u>

A typical example of a torque trace is shown in Figure 2. Isometric MVC torque was the highest measured voluntary torque (before the electrical stimulation) from the two extension contractions performed at each knee angle. Torque of stimulation on the relaxed muscle (a) was the average torque of the two stimulations applied on the relaxed muscle. Torque before stimulation (b) was used as a reference value for the torque of superimposed stimulation (c). MVC torque could not be used for this purpose, as b was not always as high as MVC torque. The maximal torque generating capacity (MTGC) of the muscle was estimated using the following formula:

$$MTGC = \frac{b}{1 - c/a} \quad \text{(de Haan et al., 2000).}$$

Voluntary activation (VA) is the ratio between MVC torque and MTGC. With respect to this technique it must be noted that the calculation of MTGC is not perfect because any antagonistic torque will lead to an underestimation of the MTGC.

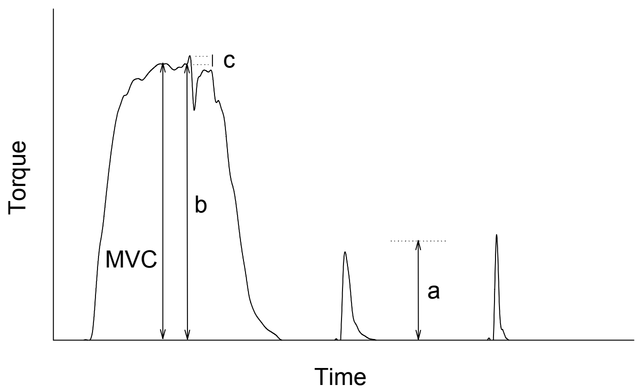

Figure 2. Example of a torque trace during isometric knee extension. MVC = maximal voluntary contraction torque, a = torque of stimulation on relaxed muscle, b = voluntary torque before stimulation, c = torque of superimposed stimulation.

To check whether the criterion used in the familiarisation trial (50% dynamic torque decline) has lead to the desired degree of fatigue, the mean peak torque of the last three concentric contractions of each protocol was compared with the mean peak torque of the first three contractions of the first protocol. In addition, the isometric flexion torque before and after the exercise (of familiarisation session) was compared.

EMG measurements

Average rectified value (EMG_{ar}) of the BF EMG was determined during maximal extension and flexion contractions. Initially, the time point of the highest average torque over 500 ms was calculated. From this instant, the EMG_{ar} was determined over a time interval of 500 ms (Merletti et al., 1999). For extension contractions (on which a stimulation was applied) this interval was always before the superimposed stimulation to prevent that EMG_{ar} was determined during the stimulation.

The antagonist EMG_{ar} during extension contractions at each knee angle was normalised as a percentage of the BF EMG_{ar}, at the same angle, during a maximal flexion contraction (Kellis and Baltzopoulos, 1996b).

Statistical analysis

All results are expressed as mean (± SD). Non-parametric statistics was used, as the number of subjects in some conditions was limited. To investigate the effect of the fatigued hamstring muscle on EMG_{ar}, MVC, MTGC and VA, Wilcoxon signed rank tests were used. A Friedman test was used to investigate the effect of joint angle on EMG_{ar} before and after fatigue. This latter test was also used to examine the decline in isometric and dynamic flexion torque after the dynamic protocol. The level of statistical difference was set at $P<0.05$. This P value is used in all chapters of this thesis. Actual P values are provided if they were close to 0.05.

RESULTS

Due to different technical problems (i.e. disconnected electrode, unsaved data) some measurements were found to be missing. Therefore, the number of subjects for MVC, MTGC, VA and EMG_{ar} varies in the different conditions. The exact numbers of subjects are given in parentheses for each variable and condition in Table 1 and Figures 3-6 if they were less than 7.

Hamstring fatigue

The mean concentric and isometric flexion MVC values were lowered by an average of 50 and 64 Nm (Table 1). The mean number of concentric flexion contractions required to induce fatigue in the four protocols was 52 ± 6, 42 ± 10, 52 ± 6 and 43 ± 9, respectively. These produced a mean decline in flexion torque of 53 ± 10%, 48 ± 16%, 45 ± 10% and 48 ± 6%, respectively, which was not different for the four protocols ($P>0.05$). The reduction in isometric flexion torque determined in the practice session was 48 ± 20% and this was not different from the decline in dynamic flexion torque ($P>0.05$).

Table 1. Concentric and isometric knee flexor torque (Nm) before and after the concentric fatigue protocol.

	Protocol 1	Protocol 2	Protocol 3	Protocol 4	Isometric
Pre	100.3 ± 19.9	-	-	-	118 ± 31
Post	47.1 ± 11.5	49.5 ± 8.9	54.6 ± 13.1	51.8 ± 11.2	54 ± 19

Data are presented as mean ± SD. N = 7, except for the post-isometric condition (n = 5). Values of protocol 1-4 are of 4 fatigue protocols in the experimental session, while isometric torque values are from the familiarisation session.

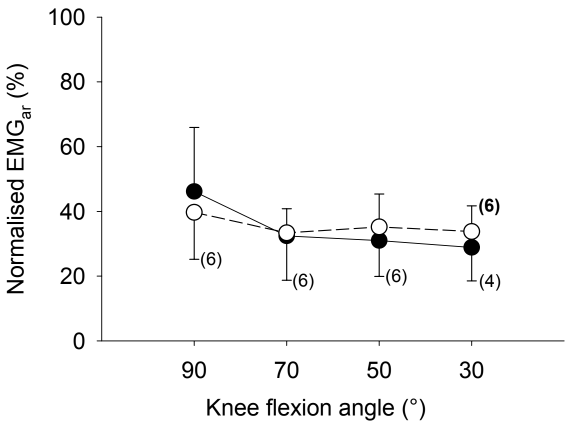

Figure 3. Antagonist biceps femoris EMG (BF EMG$_{ar}$) recorded during maximal isometric extension contractions, before (•) and after (○) fatiguing exercise. BF EMG$_{ar}$ is normalised to the EMG$_{ar}$ during a maximal flexion contraction at the same knee angle. If the number of subjects deviates from 7 the exact number is provided in parentheses (in bold for pre-fatigue condition).

Antagonist activity

The knee flexion angle did not significantly affect antagonist EMG_{ar} during maximum extension contractions before fatigue (Figure 3). Mean normalised EMG_{ar} before fatigue was 35 ± 14%. The fatiguing protocol did not significantly change EMG_{ar}, with the mean normalised EMG_{ar} measured as 36 ± 14%, indicating that the neural drive to the hamstring muscle was maintained.

Maximal voluntary contraction torque

Fatiguing the antagonists did not significantly affect the MVC torque at any of the knee angles. The mean MVC torque before fatigue was 226 ± 69 Nm and after fatigue 211 ± 60 Nm (Figure 4). The mean and SD of this difference was 19 ± 35 Nm.

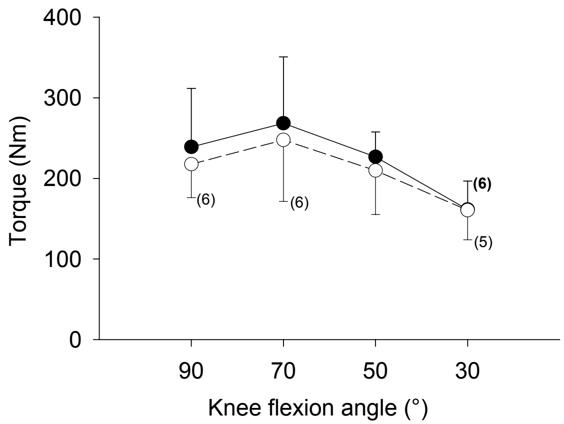

Figure 4. Maximal voluntary contraction (MVC) torque of the extensor muscles before (•) and after (○) fatiguing exercise as a function of knee angle. Values are means ± SD. If the number of subjects deviates from 7 the exact number is provided in parentheses (in bold for pre-condition).

Maximal torque generating capacity

Similar to the observations of MVC torque, fatiguing the hamstring muscle had no significant effect on the MTGC of the knee extensors at any of the knee angles. The mean MTGC before exercise was 244 ± 74 Nm and after exercise 253 ± 73 Nm (Figure 5).

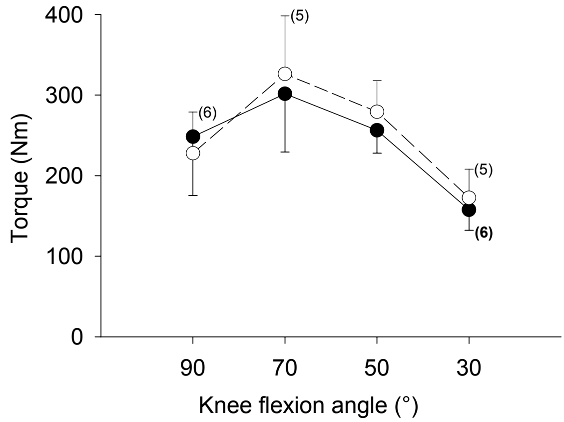

Figure 5. Maximal torque generating capacity (MTGC) of the extensor muscles before (•) and after (○) fatiguing exercise as a function of knee angle. Values are means ± SD. If the number of subjects deviates from 7 the exact number is provided in parentheses (in bold for pre-condition).

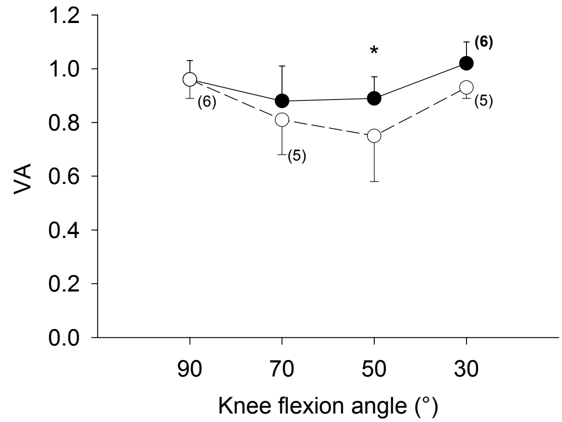

Figure 6. Voluntary activation (VA) of the extensor muscles before (•) and after (○) fatiguing exercise as a function of knee angle. Values are means ± SD. If the number of subjects deviates from 7 the exact number is provided in parentheses (in bold for pre-condition). *VA at 50° knee angle was significantly lower post-exercise (P=0.018).

Voluntary activation

At a knee joint angle of 90° the mean activation of the knee extensors was shown to be 97% and 96% of maximum before and after the fatiguing exercise, respectively (Figure 6). At 70° knee flexion the level of activation pre- and post-fatigue was 88 and 81%, respectively (P>0.05). At 30° knee flexion these values were 102 and 93% (P>0.05). Only at a knee joint angle of 50° was there a significant reduction in VA from 89% to 75% (P=0.018) after the fatiguing exercise.

DISCUSSION

The main finding of the present study was that a fatigued hamstring muscle did not result in an increase in net extension torque. In addition, there was no significant effect of knee angle on antagonist activation. Thus, neither of our hypotheses was supported. However, it must be kept in mind that, in some conditions, the number of subjects was small which makes it difficult to make generalised conclusions.

Extension MVC torque

The measured EMG_{ar} of the BF during extension contractions was 30-40% of that observed during a flexion MVC. These values are in agreement with antagonist contribution reported in the literature (Aagaard et al., 2000a; Aagaard et al., 2001; Kellis and Baltzopoulos, 1996b; Kellis and Baltzopoulos, 1998; Kellis and Kellis, 2001). Antagonist torque could be estimated from an EMG-torque relationship of the hamstring muscle (Baratta et al., 1988; Kellis and Baltzopoulos, 1997). However, activation of the quadriceps muscle may alter the EMG-torque relationship in the hamstring muscle.

Despite these high antagonist levels in our study, a ~50% reduction in antagonist dynamic torque production had no discernible effect on net isometric MVC extension torque. Theoretically, four possible mechanisms can be postulated to explain this unexpected result.

It could be argued that the dynamic protocol has, to some extent, fatigued the quadriceps muscle, as this muscle was an antagonist during the flexion movement. If this was the case the MTGC of the quadriceps muscle would have declined. However, the MTGC of the extensor muscle was not affected after the fatiguing protocol (Figure 5) and thus it can be concluded that no fatigue had occurred in the quadriceps muscle. This rules out this first possible explanation.

Another hypothesis for not finding an increased extension MVC torque could be that different fibre populations of the hamstring muscle group were fatigued in the dynamic protocol than recruited in the extension contractions, as also suggested by

Kellis and Kellis (2001). High intensity voluntary dynamic exercise selectively fatigues fast, fatigable muscle fibres (Beelen and Sargeant, 1991). After 30 s of maximal cycling exercise, greater phosphocreatine (PCr) and ATP depletion is observed in pools of type II fibres compared to type I fibres (Casey et al., 1996). Karatzaferi et al. (2001b) reported that, after 10 s of maximal cycling exercise, micro-dissected single type II fibres had a greater reduction of PCr than type I fibres. Concurrent with this decline in PCr, associated changes in ATP and IMP were found in the respective fibre populations. Another study by this group (Karatzaferi et al., 2001a) showed that after 25 s of cycling, there were further decreases of PCr and ATP and increases in IMP, these being greater in type II than type I fibres. Though the task performed in those experiments differs from the present study, our opinion is that the general conclusion can be the same. Therefore, we assume that, in our bout of maximal dynamic contractions, mainly fast, fatigable muscle fibres were fatigued. However, during maximal extension contractions the antagonist hamstring muscle was performing submaximal isometric contractions, as evidenced by the EMG. In submaximal exercise, mainly slow, non-fatigable motor units would be expected to be recruited. Thus, during the coactivation contractions a separate population of muscle fibres of the hamstring muscle was recruited rather than those, which were fatigued by the dynamic flexion protocol.

To our knowledge, this is the first study in which the influence of fatigued antagonists on knee extension torque has been investigated. Bigland-Ritchie et al. (1995) reported that one potential problem in addressing fatigue is the exercise employed to generate fatigue. We employed a dynamic fatiguing protocol but measured net extension torque under isometric conditions. As no information was available on the effect of hamstring fatigue on knee stability, and as isometric extension has a smaller risk factor than dynamic contractions we have chosen to test isometrically. It has been shown that torque declines more in dynamic contractions compared with isometric contractions. For example, James et al. (1995) reported that there was a 25% greater loss in dynamic isokinetic force than in isometric force during a bout of dynamic knee extensor exercise. A similar finding had previously been reported by Jones (1993) who found that, following repeated bouts of dynamic fatiguing exercise, the reduction in power output was 30% greater than the decline in isometric force. Therefore, it would be possible that the experimental protocol explains the absence of an increase in net extension torque after fatigue. However, in the present study the ~50% decline in isokinetic flexion torque development was mirrored by a decline in isometric flexion torque of approximately the same magnitude.

Theoretically, with a fatigued hamstring muscle, it could be expected that the neural drive to the quadriceps muscle would be reduced to prevent high shear forces in the knee joint. Such an inhibition was not found at 90° knee angle as there was no evidence of decreased activation level at that knee angle (~95% before and after fatigue), but at the other knee angles there was a decrease in VA of between 8-15%, which was significant at 50° knee angle (Figure 6). It is possible that this decline in activation has masked the effect of hamstring fatigue on MVC extension torque.

Effect of knee angle

We expected that the antagonist activity would vary with the knee joint angle, with higher activity in the more extended positions as at those positions the strain on the ACL is greatest (Beynnon et al., 1992; Hirokawa et al., 1992; Kaufman et al., 1991; More et al., 1993; Nisell et al., 1989; Renstrom et al., 1986). However, based on the measured EMG_{ar}, knee angle had no effect on hamstring activation level (Figure 3). This result is in contradiction to the generalised idea that antagonist activity of the hamstring muscle is regulated by a protective neural reflex (Solomonow et al., 1987) which is mediated by strain on the ACL. However, our results are in line with the work from the group of Grabiner (1989; 1992) as we were also not able to provide evidence for an automatic reflex between ACL strain and hamstring activity.

Antagonist activity

The finding that antagonist BF EMG after fatigue was not significantly different from the values before fatigue is consistent with the results of Kellis and Kellis (2001) and Miller et al. (2000). In their study, Kellis and Kellis (2001) fatigued both quadriceps and hamstring muscles using a reciprocal isokinetic fatigue test to examine the antagonist EMG values during fatigue. The mean fatigue of the hamstring muscle after the exercise protocol was comparable with the present study, 49%, but the antagonist EMG values of the BF remained the same during the test. In the study of Miller et al. (2000) there was also no significant decline in BF root mean square (RMS). The degree of fatigue achieved in this study was not reported but the subjects completed 30 extension/flexion repetitions at $300°\cdot s^{-1}$ and thus it could be expected that the decline in torque would be comparable with the present study.

Conclusion

Fatiguing the hamstring muscle group under the present experimental conditions has no effect on net extension MVC torque. In addition, no angle-dependent effect of antagonist activity could be demonstrated. The primary finding might be explained by (1) fatigue of predominantly fast fibres, which are hardly recruited during the antagonist activation, (2) a greater loss in dynamic torque compared to isometric torque and/or (3) a combination of a reduction in antagonist torque and quadriceps voluntary activation. The implication of this finding is that, during *in vivo* exercise, appropriate coactivation and co-ordination around the joint may be maintained, despite exercise induced fatigue of an antagonist muscle group.

ACKNOWLEDGEMENTS

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CHAPTER 3

Changes in PCr/Cr ratio in single characterized muscle fibre fragments after only a few maximal voluntary contractions in humans

ABSTRACT

Aim: This methodological study investigated the number of brief maximal voluntary isometric contractions (MVC) needed to show evidence of fibre activation, as indicated by changes in the phosphocreatine to creatine (PCr/Cr) ratio. **Methods:** Subjects performed series of 4, 7 and/or 10 maximal voluntary isometric contractions (1 s on, 1 s off) of the m. quadriceps (60° flexion angle). Needle biopsies of the m. vastus lateralis were taken at rest and immediately post-exercise. Single muscle fibres were dissected from the freeze-dried samples and classified as type I, IIA or IIAX, using mATPase stainings. Fragments of characterized fibres were analysed for PCr and Cr content. Analyses of variance were performed to investigate changes in PCr/Cr per fibre group over time, followed by Bonferroni post-hoc test ($P<0.05$). The 5^{th} percentile of resting values of each fibre group was determined. **Results:** Mean PCr/Cr ratios after 4, 7 and 10 MVCs were significantly lower for all fibre groups ($P<0.05$). The mean decreases were 44, 64 and 76%, respectively. However, only after 7 and 10 contractions PCr/Cr ratios of all, but 3 type I and 2 type IIAX fibres, individual fibres were below the 5^{th} percentile. **Conclusion:** In very short duration exercise, involving 7 brief maximal voluntary contractions, changes in the PCr/Cr ratio indicated activation of different characterized muscle fibre fragments. The results suggest that this approach may be useful for investigating the pattern of fibre type activation in exercise of very short duration.

INTRODUCTION

Muscle force can be regulated by changing the number of activated motor units and/or by changing the firing rate of the activated motor units that is by rate coding. Motor unit recruitment patterns have been investigated using EMG from individual motor units during isometric contractions (e.g. Desmedt and Godaux, 1977a; Grimby and Hannerz, 1977; Milner-Brown et al., 1973). However, the application of this technique using needle electrodes is difficult and very limited during dynamic contractions.

As an alternative, depletion of muscle glycogen has been widely used as an indication of fibre activation in humans (e.g. Vøllestad and Blom, 1985; Vøllestad et al., 1992; for a review see Saltin and Gollnick, 1983). Those studies have most often used whole body exercise, such as cycling, of relatively long duration lasting for at least 10 min (Gollnick et al., 1973a; Vøllestad et al., 1984; Vøllestad and Blom, 1985). Glucose changes have been investigated during supramaximal exercise of shorter duration (30 s – 3 min) but this study aimed to compare rates of glycogenolysis and not fibre type recruitment (Vøllestad et al., 1992). There are, however, difficulties in both measuring and interpreting changes in muscle fibre glycogen concentration in relation to contractile activity, not least because not all of the decrease in glycogen necessarily leads to ATP synthesis. Moreover, glycogenolysis has been shown to be stimulated by adrenaline in human muscle type I fibres during contraction (Greenhaff et al., 1991) and glycogen loss may even occur in non-exercising muscle (McDermott et al., 1987). In addition, other substrates are available in the muscle cell, such as glucose, fatty acids and triglycerides and different muscle fibre types will use glycogen at different rates and in different proportion relative to the other available substrates (Essén, 1978; Kernell et al., 1995). A further limitation is that, due to the relatively high concentration of glycogen in human muscle fibres, detectable changes are only seen after some minutes of exercise.

Using a method previously developed (Sant'Ana Pereira et al., 1995b) and improved in our laboratory (Karatzaferi et al., 1999), it is now possible to measure changes in phosphocreatine (PCr) and creatine (Cr) in single characterized muscle fibre fragments (type I, IIA and IIAX) isolated from a needle biopsy of human muscle after exercise lasting only a few seconds. The advantage of this method over glycogen depletion detection is that a decrease in PCr is directly linked to ATP resynthesis during exercise. Therefore, this parameter can be used as a marker for muscle fibre activation (Conjard and Pette, 1999).

The present study was designed to develop a protocol to allow investigation of fibre activation in several exercise modes and with different intensities of brief duration exercise, using the decrease in PCr. The changes in the ratio of PCr/Cr were used as an indication of activation. Thus, the purpose of this study was to determine whether substantial changes in the PCr/Cr ratio are induced in a small number of maximal voluntary contractions. We hypothesised that a brief exercise of only 4 to 10 maximal contractions of 1 s duration is enough to be able to ascertain that a fibre of a specific type has been activated.

METHODS

Subjects

Seven generally active subjects (4 males, 3 females) were studied. Male subjects were denoted as A, B, C and E, while female subjects were D, F and G. Mean ± SD age, height and body mass were 30 ± 5 years, 189 ± 6 cm and 84 ± 12 kg for the male subjects and 23 ± 6, 169 ± 6 and 69 ± 3 for the female subjects. None of the subjects had a history of muscle or metabolic diseases. Subjects gave informed consent in accordance with the procedures required by the ethical committee of the Vrije Universiteit Medical Centre that approved the study.

Experimental protocol

Subjects performed one or three series of maximal voluntary isometric contractions (MVC) of the knee extensors in a seated position with a hip angle of 75° and a knee angle of 60° (0° = full extension). Straps were applied to the lower leg, pelvis and torso to stabilise the subject. Each series of contractions consisted of 4, 7 or 10 MVCs of 1 s duration (1 s rest). Rest between series was at least 10 min. To impose the 1 s on/1 s off rhythm, an auditory signal was given during the contraction phase. Subject C and D only performed the series of 7 MVCs. All subjects were verbally encouraged to make a maximum effort and the number of performed contractions was loudly called. Before collection of experimental data, the subjects came to the laboratory for a practice session.

Torque analysis

For each series of contractions, mean torque was expressed relative to the maximal torque measured. This relative torque was subsequently integrated over time (torque time integral in arbitrary units). Exercise time was determined for each contraction (period in which active torque was produced).

Muscle biopsy

Before beginning the experiment, two small incisions were made in each leg (at one-third distal of the distance between the lateral femoral epicondyle and trochanter major) after local anaesthesia (2% lidocaine) of the skin and fascia. Immediately following the last contraction of a series, a muscle sample was collected from the m. vastus lateralis of the exercised leg using a Bergström type biopsy needle with suction (depth 2-3 cm measured from the skin). In addition, from one leg a resting sample was obtained. As two biopsies were taken from one leg the needle was directed either proximally or distally. Subject E was included in the study for a resting sample only. Upon removal from the muscle, the sample was immediately frozen in liquid nitrogen and freeze-dried overnight. Biopsies were frozen within ~4-9 s from end of exercise. The freeze-dried samples were stored desiccated in tubes sealed with laboratory film. Each tube was placed in another small jar with some silica gel, sealed with laboratory film and stored in liquid nitrogen vapour until analysed.

Individual fibre fragments of 2-3 mm length (100-160 from each sample) were dissected under conditions of controlled ambient temperature and relative humidity (20-25°C and <35% RH). Each fibre fragment was divided into two parts, one for histochemistry and the remaining part for analysis of metabolites. The methods used for single fibre typing and muscle metabolite analysis are extensively described elsewhere (Karatzaferi et al., 1999; Sant'Ana Pereira et al., 1995b).

Some samples were excluded from the study due to poor quality of some samples or because of a too long freezing time (>~10 s). Refer to Table 1 for information on available samples and number of fibres characterized and analysed for PCr and Cr for each subject.

Table 1. Muscle samples and number of fibres analysed for PCr and Cr of each subject.

Subject	Type	Rest	4 MVCs	7 MVCs	10 MVCs
	I	19[1]	19[2]	15[4]	20[3]
A	IIA	11	20	19	11
	IIAX	19	9	8	5
	I	18	20		
B	IIA	14	15		
	IIAX	29	2		
	I	20		20	
C	IIA	25		19	
	IIAX	7		14	
	I	17		19	
D	IIA	9		20	
	IIAX	10		9	
	I	14			
E	IIA	10			
	IIAX	20			
	I			26	
F	IIA			14	
	IIAX			29	
	I				32
G	IIA				6
	IIAX				2

[1,2,3,4] gives the order in which the muscle samples were collected from subject A.

Histochemistry

Serial sections (~10 µm) of gelatine embedded single fibre fragments were cut and characterized for acid mATPase stability at pH 4.6 and 4.4 (adapted from Brooke and Kaiser, 1970). An image analysis system was used to measure the optical density (OD) of each fibre fragment (Sant'Ana Pereira et al., 1995b). Based on the OD values from ATPase, pre-incubation pH 4.4, the fibres were classified into type I and II fibres. The ATPase, pre-incubation pH 4.6, was then used to divide type II fibres in

IIA and IIAX fibres. The lightest fibres were classified as type IIA (0% IIX isoform) and the darkest fibres as type IIX (100% IIX isoform). OD values between 0 and 100% were divided into two categories, type IIA (0-15% IIX) and type IIAX (15-100% IIX)(Karatzaferi et al., 2001b). Originally, three groups were distinguished, type IIA, IIAx (15-50% IIX) and IIXa (50-100% IIX) but because of the limited number of IIXa fibres, type IIX containing fibres were combined into one group, IIAX fibres.

Analysis of metabolites

From the characterized muscle fibre fragments of each muscle sample, a maximum of ~20 fibres from each fibre type were analysed for phosphocreatine (PCr) and creatine (Cr) by using reverse-phase high-performance liquid chromatography (HPLC) with ultra-violet photometric detection (Karatzaferi et al., 1999) following overnight extraction in 60% methanol (Donofrio et al., 1978).

Statistics

Analyses of variance were used to test for significant differences in the PCr/Cr ratio per fibre group over time. If significant effects were observed, Bonferroni post-hoc tests were performed. As the PCr/Cr ratio of the different fibre types for the different conditions was in general normally distributed it was allowed to use parametric statistics. Because of the relatively small number of fibres for some conditions, it could also be argued to use non-parametric statistics. Since the parametric statistical analysis is more stringent than the non-parametric analysis, any significance detected with the first would also give significance with the latter. Of each fibre group at rest, the 5^{th} percentile was determined and taken as the minimal resting value. Further, metabolic data of individual fibres are shown. The level of significance was set at $P<0.05$.

RESULTS

Torque

The mean normalised torque time integral per contraction was not different (78, 72 and 78 a.u.) for the series of 4, 7 and 10 contractions, respectively, as was the mean exercise time per contraction (1.18, 1.07 and 1.15 s, respectively). Thus, the intensity of the contractions did not differ between the series. Refer to Table 2 for individual values.

Table 2. Mean torque time integral and exercise time per contraction.

No. of contractions	Torque time integral (a.u.)				Exercise time (s)			
4 (A, B)	82.8	73.5			1.2	1.2		
7 (A, C, D, F)	76.4	70.9	76.1	66.4	1.2	1.2	1.0	0.9
10 (A, G)	91	65.8			1.3	1.0		

Individual data of the different subjects. Characters between parentheses are the concerning subjects.

PCr/Cr ratio

Figure 1 shows the PCr/Cr ratio for the 7 subjects separately.

Rest

The mean values at rest were respectively 2.2, 1.9 and 2.0 for type I, IIA and IIAX fibres. The 5th percentile values at rest for these fibre groups were 1.6, 1.3 and 1.3, respectively.

Exercise

After 4 contractions, the mean PCr/Cr ratios were 1.4, 1.1 and 1.0 for type I, IIA and IIAX fibres, respectively. The mean ratio had declined significantly in all fibre groups ($P<0.05$); 37, 44 and 51%, respectively. A further significant reduction, compared to resting values, in the mean PCr/Cr ratio was present after 7 and 10 contractions ($P<0.05$). After 7 contractions, the mean values were 1.0, 0.6 and 0.6. (reductions of 56, 67 and 68%) in type I, IIA and IIAX fibres, respectively. The mean values after 10 contractions were 0.7, 0.4 and 0.4 for type I, IIA and IIAX fibres, with reductions of 67, 81 and 81%, respectively compared to resting values.

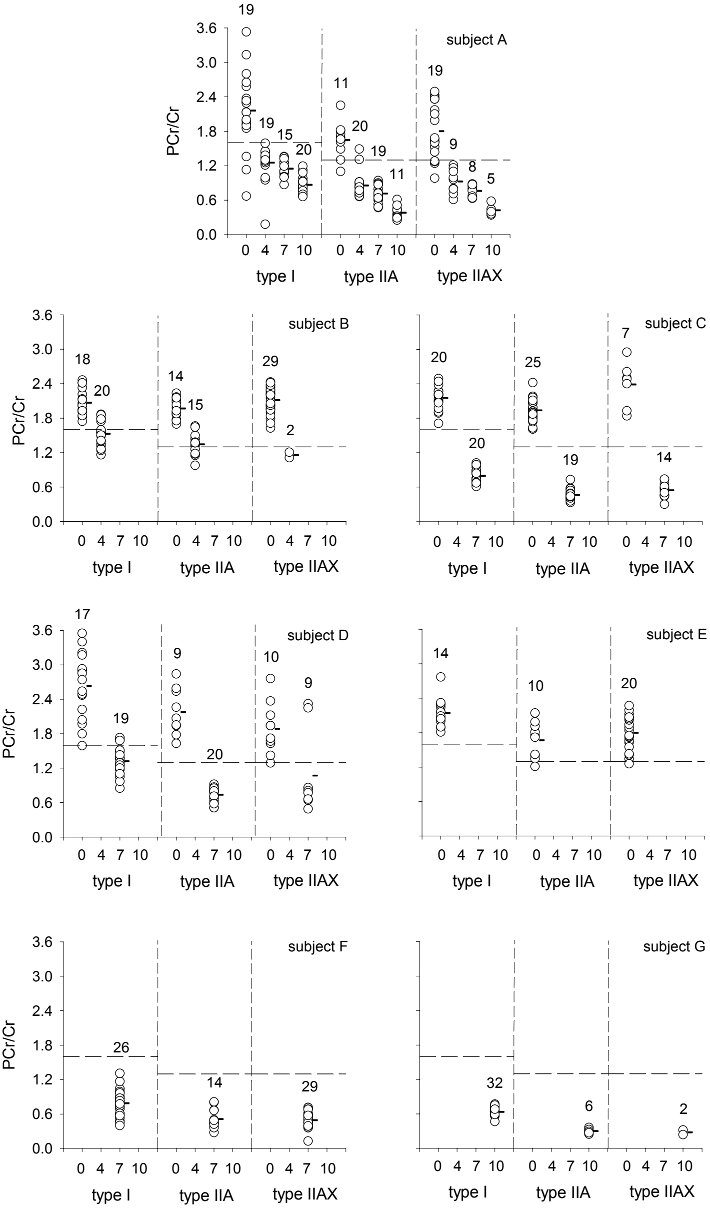

Figure 1. Single fibre PCr/Cr ratio in different fibre types at rest and after series of contractions. Subject A shows data for rest and all series (4, 7 and 10 contractions). The remaining subjects show data for rest and/or one of the series. Total number of fibres analysed for each fibre type and condition are also shown. The mean values for groups of fibres are shown by ▬. For the three fibre groups, the 5th percentile of resting values of all subjects is shown by the dashed line (----).

The data of the single fibre fragments indicate that after 4 MVCs, respectively 87 and 66% of all type I and IIA fibres showed ratios below the 5^{th} percentile of resting values. Of the type IIAX fibres, all ratios were below this value. However, after 7 and 10 contractions, only 3 type I (4%) and 2 type IIAX (3%) fibres after 7 MVCs of one subject (D) had a PCr/Cr ratio, which was still higher than the 5^{th} percentile of the resting values. All the remaining fibres had ratios, which were lower than the 5^{th} percentile of the concerning fibre group.

DISCUSSION

The present study shows that changes in the PCr/Cr ratio can be used to detect muscle fibre activation after a minimum of ~7 maximal voluntary contractions.

Analysis of muscle samples

The preparation of single fibre fragments from the dried samples, characterization of the cross-sectioned fibre fragments and HPLC analysis of the remaining fragments is an extremely time consuming process. Therefore, only 7 subjects were included in the study. After characterizing single fibre fragments, a total of 615 fibres were analysed for metabolites from 13 samples. Unfortunately, data from some samples were lacking because their quality was poor so that no fibres could be dissected from the samples. In addition, in some cases the total time from end of exercise to freezing exceeded ~10 s. As the half-time of PCr resynthesis is ~30 s (Harris et al., 1976; Kushmerick and Meyer, 1985; Sahlin et al., 1979) it could be expected that PCr had recovered to a relatively large extent. Data of such biopsies would decrease the sensitivity of the method and were therefore excluded from the study.

PCr/Cr ratio

Instead of using the decrease in PCr in single (rabbit) fibres (Conjard and Pette, 1999) we have used the ratio of PCr to Cr as a marker for activation. There are several reasons for choosing the PCr/Cr ratio. First, the PCr/Cr ratio is directly related to the ATP/ADP ratio, known as an indicator of the energetic state of the ATP system (Conjard et al., 1998). Thus, the single fibre ratio of PCr to Cr can be used as a representation of the energy state of the fibre. Second, when using a decrease in PCr content it is necessary to weigh each fibre fragment to be able to express the PCr content per unit dry weight. Experience from previous analyses has proven that it is extremely difficult to reliably weigh single muscle fibre fragments, which are in the range of 1-3 μg. Errors in weighing are then reflected in the PCr concentration. Alternatively, the measured PCr should be normalised for the total Cr concentration of the subjects fibre types (Harris et al., 1976; Sabina et al., 1983; Sahlin et al., 1997; Stathis et al., 1994). However, by using this method, PCr concentrations are much more subjected to differences between subjects than the PCr/Cr ratio because of variation in total creatine per subject. Third, the methodological error of analysing PCr/Cr ratio within a fibre is very small because measurements of PCr and Cr are performed in the same HPLC run (Karatzaferi et al., 1999). Fourth, the ratio is more sensitive to changes as, because total creatine in a fibre before and after exercise is always constant (Sahlin et al., 1997; Soderlund and Hultman, 1991; Tesch et al., 1989), a decrease in PCr implies an increase in Cr. So, besides a more sensitive measure, the ratio is also less subjected to measurement errors and individual differences than the absolute PCr content.

Rest

In earlier studies, metabolites in whole muscle samples (that is, a homogenate of the muscle fibre types present) were measured (Edstrom et al., 1982; Sahlin et al., 1997; Stathis et al., 1994; Tesch et al., 1989). Mean PCr/Cr ratios calculated from these PCr and Cr contents varied between 1.6 and 2.2. So, our mean value of the PCr/Cr ratio of all fibre groups (2.0) is consistent with previous data on mixed muscle. Karatzaferi et al. (1999), using the same method as in the present study, demonstrated PCr/Cr ratios in type I fibres of 1.6 and 2.1 in an endurance trained athlete and a not specifically trained subject, respectively. The ratios in type II fibres were 1.2 and 2.2, in both subjects, respectively. From resting data of another study of our group (Karatzaferi et al., 2001b), PCr/Cr ratios can be calculated to be 2.3, 2.0 and 1.9, respectively in type I, IIA and IIAX fibres. Thus, the present data of the three fibre groups (2.2, 1.9, 2.0, respectively) demonstrate ratios that are comparable with previous data of our group (Karatzaferi et al., 1999; Karatzaferi et al., 2001b).

Exercise

At fatigue, the PCr/Cr ratio of a mixed-fibre muscle decreased by ~83% of the values at rest to ~0.3 (Sahlin et al., 1997; Stathis et al., 1994; Tesch et al., 1989). It is difficult to make a direct comparison of this exercise data, however, due to the difference in exercise type and protocol. Karatzaferi et al. (2001b) measured PCr and

Cr levels in single muscle fibres after 10 s maximal dynamic exercise on an isokinetic cycle ergometer. Their data show reductions in type I, IIA and IIAX fibres to 0.6, 0.4 and 0.3 in type I, IIA and IIAX fibres, respectively. All these data are similar or slightly lower than the ratios obtained after 10 contractions in the present study (0.7, 0.4 and 0.4).

Detection of fibre activation

In this study we considered that fibres showed evidence of activation if, as a consequence of the series of (4, 7 or 10) contractions, the PCr/Cr ratio fell below the 5^{th} percentile of the resting values for the same fibre type. The 5^{th} percentile values for type I, IIA and IIAX fibres were respectively 1.6, 1.3 and 1.3 (Figure 1, dashed horizontal lines).

In the present study we were concerned to identify evidence of activation in the isolated fibre fragments. We therefore set rigorous criteria as the threshold for accepting that a characterized fragment had been activated. Clearly any resynthesis of PCr during or after exercise may lead to an underestimate of the level of activation among the analysed and characterized fibre fragments. Nevertheless, the results do indicate that, in a very few contractions, reductions in the PCr/Cr ratio were detectable in nearly all fibre fragments examined compared to the resting values for the same fibre type. We believe that the technique described may prove a valuable approach for the study of fibre type activation patterns in very short duration exercise.

In high intensity (100% MVC) short-term exercise it can be expected that all fibres are activated. After only 4 contractions there was a significant decline in the mean PCr/Cr ratio for all fibre types. However, data from individual fibres show that not all fibres demonstrate ratios below the 5^{th} percentile level of the resting value for that fibre type. From the three fibre groups, only IIAX fibres showed values, which were all below the 5^{th} percentile value. After 7 contractions there was a further significant decline in the mean ratio. When individual fibre data are inspected it appears that the ratio of all fibres fragments analysed were below the 5^{th} percentile value. Only 3 type I (4%) and 2 type IIAX (3%) fibres showed a ratio, which was higher. Thus, the present results have shown that a series of ~7 maximal contractions will generate sufficient changes in the PCr/Cr ratio to indicate activation of different muscle fibre types. It must be kept in mind though, that the number of biopsies available of all conditions was limited and there was some variability between subjects. This variability is, however, consistent with previous literature that shows large variations in PCr content between subjects and between fibres, even within the same fibre type (Sahlin et al., 1997). This variation can mainly be explained by physiological variation (Sahlin et al., 1997) but may also be affected by the differences in time of biopsy sampling. Another source of variability, especially in resting values (for example subject A, Figure 1), is the relatively low Cr compared to PCr concentration in resting muscles. Small variations in PCr content will then have a relatively large influence on the PCr/Cr ratio measured at rest. However, as described before, the PCr/Cr ratio is less subject to variation than is the PCr concentration.

This relatively large variability, however, is also a problem using the present technique. Another problem that needs to be mentioned here is the difference between different fibre types in ATP turnover rate and the recovery in the brief period between sampling and freezing. This implies that differences in the decline of the PCr/Cr ratio between fibre types are not necessarily a result of different activation levels.

Statistical treatment

In the present study, analysis of variance was used to test for significant differences compared to resting values. For this statistical analysis the data of all 7 subjects were pooled. It was not possible to perform a repeated measures analysis, because fibres after exercise were not the same fibres as measured at rest. Moreover, not all subjects had samples taken after all series and some of the samples had to be excluded from the study. So, it should be realised that, by pooling the data of all subjects, between-subjects variation in PCr/Cr ratios is not taken into account. It could be argued that activation patterns between subjects are different. Therefore, data of all subjects are shown separately (Figure 1).

Conclusion

Previously, the study of fibre type activation patterns in short-term whole body human exercise has been problematic. With the method described here it will be possible to identify fibre types that have been activated during exercise involving only a very few contractions. We believe that, although it is time consuming and laborious, the measurement of the PCr/Cr ratio in single fibre fragments will allow the investigation of the pattern of muscle fibre activation in different types and intensities of exercise, using only a brief, initial period of the exercise.

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CHAPTER 4

Metabolically assessed muscle fibre recruitment in brief isometric contractions at different intensities

ABSTRACT

This study investigated the recruitment of type I, IIA and IIAX fibres after 7 isometric contractions at 40, 70 and 100% maximal voluntary knee extension torque (MVC, 1 s on/1 s off). Biopsies of the m. vastus lateralis were collected from 7 subjects at rest and immediately post-exercise. Fibre fragments were dissected from the freeze-dried samples and characterized as type I, IIA and IIAX using mATPase staining. Phosphocreatine (PCr) and creatine (Cr) content were measured in the remaining part of characterized fibres. A decline in the ratio of PCr to Cr (PCr/Cr) was used as an indication of activation. The mean peak torques were respectively 39 ± 2, 72 ± 2 and 87 ± 6% MVC. Cumulative distributions of type I and IIA fibres were significantly shifted to lower PCr/Cr ratios at all intensities (Kolmogorov-Smirnov test, $P<0.05$). The cumulative distribution of type IIAX fibres showed a significant leftward shift only at 87% MVC ($P<0.05$). A hierarchical order of fibre activation with increasing intensity of exercise was found, with some indication of rate coding for type I and IIA fibres. Evidence for activation of type IIAX fibres was only found at 87% MVC.

INTRODUCTION

It is generally known that, in order to produce force, motor units are hierarchically recruited, controlled by the size of the motor unit. In this scheme, often referred to as the "size principle" (Henneman et al., 1965; Henneman et al., 1974), small motor units comprised of slow fatigue resistant type I fibres are recruited at low levels of force. When larger forces are required large motor units that contain fast, fatigable type II fibres are recruited. Evidence for this concept of rank order of recruitment comes from needle or wire electrode EMG recordings in isometric contractions (De Luca et al., 1982; Desmedt and Godaux, 1977a; Freund et al., 1974; Milner-Brown et al., 1973; Person and Kudina, 1972). However, in humans mostly relatively small muscles have been studied such as first dorsal interosseus (De Luca et al., 1982; Freund et al., 1974; Milner-Brown et al., 1973) or tibialis anterior muscle (Desmedt and Godaux, 1977a). Moreover, using single motor unit EMG it is difficult to assess recruitment during contractions close to maximum due to numerous motor units firing near the electrode tip. Therefore, the present study aimed to investigate the recruitment pattern of a relatively large muscle, the vastus lateralis muscle, during submaximal and (near) maximal contractions using a new technique based on phosphocreatine utilisation in characterized single fibres.

There is also evidence for the orderly recruitment of motor units in human whole body exercise, such as cycling. With the glycogen-depletion method, using the Periodic Acid Schiff (PAS) reaction as a marker of fibre activation, it has been reported that there is a sequential depletion of glycogen in type I and type II fibres (Gollnick et al., 1973a; Gollnick et al., 1974b) or, when considering type II subgroups, type IIA and IIAX and IIX fibres (Vøllestad et al., 1984; Vøllestad and Blom, 1985). The results from Vøllestad and Blom (1985) indicate that after cycling at an exercise intensity of ~90% VO_2max, all fibres had been recruited as determined from muscle biopsies taken at the end of exercise. This is somewhat surprising as at this intensity only ~50% of the available maximum dynamic force is generated while 100% of the muscle fibres show evidence of glycogen depletion. This observation has been interpreted as indicating a very high degree of rate coding superimposed on the sequential and size related recruitment of motor units (Sargeant and Jones, 1995). Data derived from estimates of glycogen depletion as an indication of fibre activation should, however, be used with care (Kernell et al., 1995). Relatively long exercise bouts are required to elicit a measurable decrease in optical density of the fibres, which are stained for glycogen (>~10 min). Therefore, a decline in the PAS staining may be a combined effect of both intensity and duration of exercise.

Recently, we have developed a new method to assess muscle fibre activation metabolically after only a few contractions. In this method the phosphocreatine (PCr) and creatine (Cr) content of characterized single fibre fragments, dissected from vastus lateralis muscle biopsies taken at rest and immediately after exercise, is measured (Beltman et al., 2004a). Since PCr is split immediately upon muscle activation (Infante et al., 1965) the PCr/Cr ratio may be a useful indicator of fibre activation in very brief exercise.

In those experiments we have shown that a series of 7 maximal voluntary contractions leads to a decline in the PCr/Cr ratio, which is sufficient to detect activated fibres (Beltman et al., 2004a). The purpose of the present study was to investigate the activation of different muscle fibre populations (type I, IIA and IIAX) at *different intensities* (submaximal and maximal) of brief isometric exercise by measurement of PCr/Cr ratios in characterized single fibre fragments. We hypothesised that we would find an orderly recruitment of muscle fibres according to the size principle. Moreover, we expected to find differences in the decline of the PCr/Cr ratio between fibre types at the different intensities probably suggesting an effect of rate coding.

METHODS

Subjects

Seven physically active, but not specifically trained, subjects (2 female, 5 males) volunteered to participate in this study, which had approval of the ethical committee of the Vrije Universiteit Medical Centre, Amsterdam, The Netherlands and was in accordance with the Declaration of Helsinki. The mean ± SD age, height and body mass were 27 ± 6 years, 181 ± 11 cm and 80 ± 10 kg. None of the subjects had a history of muscle or metabolic diseases. All subjects gave oral and written informed consent.

Experimental protocol

Isometric knee extension torque measurements were performed on a specially designed dynamometer in a seated position with a hip angle of 75° and a knee angle of 60° (0° = full extension), which is considered to be around optimum angle for the knee extensors (Becker and Awiszus, 2001; Suter and Herzog, 1997). Straps were applied to the lower leg, pelvis and torso to stabilise the subject. In a familiarisation session, which was held on a separate day, maximal voluntary extension (MVC) torque of both legs was determined. When extension torque of maximal attempts showed no further increase this was considered to be the subject's MVC torque. For the experiment, subject A, B, C and D performed 3 series of voluntary isometric contractions of the knee extensors at different intensities. Each series consisted of 7 contractions of 1 s duration (1s rest) and was performed at three different intensities, 40, 70 and 100% MVC. The order in which the series were performed was randomised. Rest between series was at least 10 min. Subjects E, F and G only performed a series at 100% MVC. To impose the 1s on/1s off rhythm, an auditory signal was given during the contraction phase. Torque of the contractions was AD-converted (1000 Hz) and stored on disk for off-line analysis in order to determine the mean peak torque attained for each series of 7 contractions.

Muscle biopsy

With the subject on the seat of the dynamometer, small incisions were made after local anaesthesia (2% lidocaine) of the skin and fascia. Two incisions were made (one on each leg) at one-third of the distance between the lateral femoral epicondyle and the trochanter major. Muscle samples were taken at rest and immediately following the last contraction of each series from the m. vastus lateralis of the exercised leg using a Bergström type biopsy needle (Ø 5 mm, Popper Biomedical Instruments, Schuco International London Limited, London, UK) with suction. As two biopsies were taken from one leg, the needle was directed either proximally or distally. Biopsies were frozen in liquid nitrogen within ~2½-10 s (average ~5½ s) from end of exercise and freeze-dried overnight. The freeze-dried samples were stored desiccated in tubes with the lid sealed with laboratory film. Each tube was placed in another small jar with some silica gel, the lid was sealed with laboratory film and stored in liquid nitrogen vapour until analysed. Individual fibre fragments of 2-3 mm length (80-120 from each sample) were dissected under conditions of controlled ambient temperature and relative humidity (20-25°C and <35% RH). Each fibre fragment was divided into two parts, one for histochemistry in order to determine fibre type and the remaining part for analysis of metabolites. The methods used for single fibre typing and muscle metabolite analysis are extensively described elsewhere (Karatzaferi et al., 1999; Sant'Ana Pereira et al., 1995b).

Three biopsies (two resting samples and one sample at 100% MVC) were excluded from the study because, (i) no fibres could be dissected from the sample, (ii) freezing time was too long (>10s), or (iii) the resting PCr/Cr ratio of type I and IIA fibres showed values that were much lower (on average 25%) than the mean resting values of the other subjects (~2.2 and 1.9 respectively, Table 1).

Histochemistry

Serial sections (~10 μm) of gelatine-embedded single fibre fragments were cut and characterized for acid mATPase stability at pH 4.6 and 4.4 (adapted from Brooke and Kaiser, 1970). An image analysis system (IBAS, Kontron Electronik, Germany) was used to measure the optical density (OD) of histochemically treated fibre sections (Sant'Ana Pereira et al., 1995b). Based on the OD values from ATPase, pre-incubation pH 4.4, the fibres were classified into type I and II fibres. The ATPase, pre-incubation pH 4.6 was then used to divide the type II fibres into two categories, type IIA (0-15% IIX) and type IIAX (15-100% IIX, formerly called IIAB fibres)(Karatzaferi et al., 2001b; Sant'Ana Pereira et al., 1996). Of all the analysed fibre fragments the mean ± SD fibre type distribution of the different subjects was 55 ± 10% type I , 29 ± 11% type IIA and 16 ± 7% type IIAX.

Analysis of metabolites

From the characterized muscle fibre fragments of each biopsy sample, a maximum of ~20 fibres from each fibre type were analysed for phosphocreatine (PCr) and creatine (Cr) by using reverse-phase high-performance liquid chromatography (HPLC) with ultra-violet photometric detection (Karatzaferi et al., 1999) following overnight extraction in 60% methanol (Donofrio et al., 1978). The PCr/Cr ratio was used as a measure for fibre activation (Beltman et al., 2004a).

Statistics

In order to investigate the activation of type I, IIA and IIAX fibres at different intensities of exercise, cumulative distributions of PCr/Cr ratios of individual fibres were calculated, using intervals of 0.1. To be able to test for statistical significance Kolmogorov-Smirnov one-sided two-sample tests were performed on the cumulative distributions (rest compared to 39%, 39% compared to 72% and 72% compared to 87% MVC). This test detects differences in both the location and the shape of the distributions (Siegel, 1956). The level of significance was set at $P<0.05$.

RESULTS

Torque

The mean peak torque achieved was 39 ± 2, 72 ± 2 and 87 ± 6% MVC at the target intensities of 40, 70 and 100% MVC. In the remainder of this paper the contraction series are referred to by reference to the mean torque attained rather than the target.

PCr/Cr ratio

Table 1 shows the mean ±SD PCr/Cr ratio of all analysed fibre fragments for each fibre type population at rest and after exercise at 39, 72 and 87% MVC. Cumulative distributions of the PCr/Cr ratio in single fibre fragments for each fibre type at rest and after the exercise at the three different intensities are presented in Figure 1.

Table 1. PCr/Cr ratio of all analysed fibre fragments for each fibre type population at rest and for the different intensities of exercise.

Type	Rest	39%	72%	87%
I	2.2 ± 0.5 (88)	1.6 ± 0.4 (91)	1.4 ± 0.3 (64)	1.0 ± 0.3 (109)
IIA	1.9 ± 0.3 (69)	1.7 ± 0.3 (78)	1.2 ± 0.4 (65)	0.7 ± 0.2 (99)
IIAX	2.0 ± 0.4 (85)	1.8 ± 0.5 (19)	1.7 ± 0.4 (52)	0.8 ± 0.4 (81)

Data are presented as mean ± SD. Values between parentheses are the number of fibres analysed.

<u>39% MVC</u>

After 7 contractions at 39% MVC, the cumulative distribution of type I fibres showed a significant shift to lower PCr/Cr ratios ($P<0.05$). The shift in the cumulative distribution of type IIA fibres was also significant ($P<0.05$). However, this displacement is smaller compared to the type I fibres (Figure 1A and B respectively). This can also be seen in the mean PCr/Cr ratios as the decline in type I

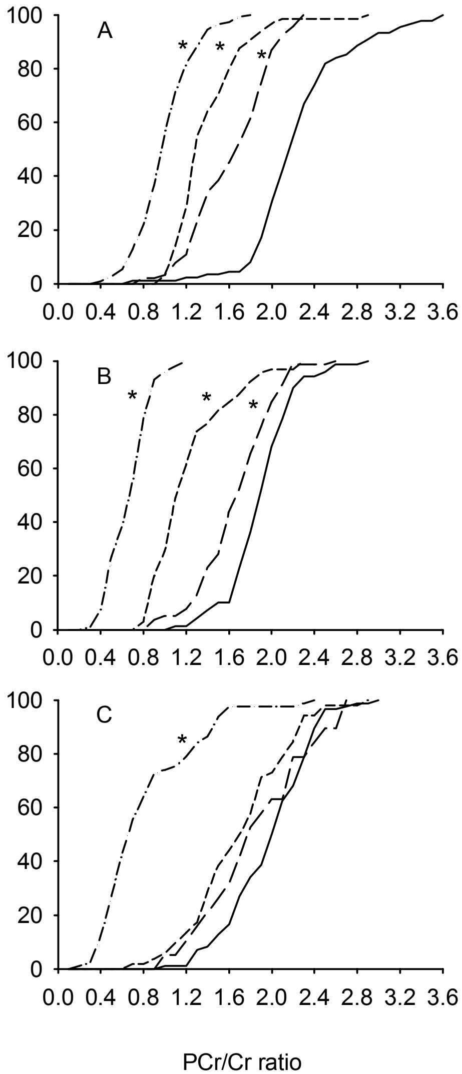

Figure 1. Cumulative frequency distribution of single fibre PCr/Cr ratios in type I (A), IIA (B) and IIAX (C) fibres at rest (——) and after series of 7 contractions at 39 (— —), 72 (---) and 87% (— · —) MVC. *Indicates significant difference of the distribution compared to rest (for 39% MVC) or the lower intensity (for 72 and 87% MVC)($P<0.05$).

fibres is 27%, while this is only 11% for type IIA fibres (Table 1). In contrast to the type I and IIA fibres, the cumulative distribution of the type IIAX fibres was not significantly different from that at rest (Figure 1C).

<u>72% MVC</u>

After exercise at 72% MVC the cumulative distribution of the type I fibres was shifted further leftwards compared with the distribution at 39% MVC ($P<0.05$, Figure 1A). Similarly, there was a further leftwards shift of the cumulative distribution of type IIA fibres to lower PCr/Cr ratios ($P<0.05$, Figure 1B). At this intensity, the magnitude of the shift in the distribution of the type IIA fibres was greater than of the type I fibres as reflected by the mean PCr/Cr ratios (Table 1). Compared with 39% MVC the ratio in type IIA fibres was 29% lower at 72% MVC, but only 13% lower in the type I fibres. Unlike the cumulative distributions of the type I and IIA fibres, type IIAX fibres had still not changed significantly from rest.

<u>87% MVC</u>

After 7 contractions at the highest intensity (87% MVC) the cumulative distributions of both the type I and IIA fibres were again shifted to significantly lower PCr/Cr ratios compared to the distributions at 72% MVC (Figure 1A and B, $P<0.05$). There was, in addition, a marked and significant shift in the cumulative distribution of the type IIAX fibres ($P<0.05$, Figure 1C). The PCr/Cr ratios of type I, IIA and IIAX fibres were respectively 29, 42 and 53% lower compared to the ratios at 72% (Table 1).

DISCUSSION

The recruitment of type I, IIA, and IIAX muscle fibres in very short duration isometric exercise was investigated using the changes in the PCr/Cr ratio of fibre fragments isolated from needle biopsies. The results show that with this new technique recruitment of fibre populations can be assessed following only a few contractions of very short duration.

In a previous methodological study we demonstrated that after 7 maximal voluntary contractions the PCr/Cr ratios of all fibre populations had declined to values below the 5^{th} percentile of resting values. Since it can be expected that all fibre types are activated in maximal contraction it was concluded that the PCr/Cr ratio could be used to detect fibre activation after a minimum of 7 maximal contractions (Beltman et al., 2004a). In the present study, this method was used to assess the recruitment pattern of muscle fibre type populations at *different intensities* of isometric contraction.

Cumulative distributions of the PCr/Cr ratios of each fibre type were calculated (Figure 1). A significant shift in the cumulative distribution of a fibre type to lower PCr/Cr ratios after exercise was interpreted as activation of that fibre type. The present results demonstrated that after 7 contractions at 39% MVC, both type I and IIA fibres had been activated. At 72% MVC, there was a further significant shift leftwards in the cumulative distribution for both type I and IIA fibre types. At neither 39% nor 72% MVC was there evidence of type IIAX fibres being activated. It was only at the highest intensity, 87% MVC, that there was evidence of IIAX fibre activation, concomitant with a further leftward shift in the cumulative distribution of the type I and IIA fibre populations.

The finding that, with increasing intensity of exercise, fibre types are recruited in an orderly and hierarchical sequence is in agreement with earlier studies using glycogen depletion as a measure of fibre activation in dynamic exercise (Gollnick et al., 1973a; Gollnick et al., 1974a; Vøllestad et al., 1984; Vøllestad and Blom, 1985). In contrast to our study, in which only few contractions were performed, those investigators used whole body exercise (cycling) of relatively long duration (20-140 min) consisting of exercise bouts of 10-20 min. From muscle biopsies, the glycogen content was measured using the PAS staining intensity to determine whether fibres had been activated. Vøllestad and Blom (1985), for example, showed glycogen depletion in type I fibres after cycling at 40% VO_2max, while at 60% VO_2max both type I and IIA fibres had been active. At the highest intensity, 90% VO_2max, type I, IIA, IIAX and IIX fibres had been active.

Although the present results obtained during brief isometric contractions are not precisely comparable, they do highlight a difficulty in interpreting the results of the earlier studies, which assessed recruitment pattern on the basis of glycogen depletion. Vøllestad and Blom (1985) showed that *all* fibre types had been activated during 25-35 minutes of exercise at 90% VO_2max. However, at this intensity the produced force is only ~50% of maximal available dynamic force at that velocity of contraction. This would suggest a very high degree of rate coding must have occurred rather than pure simple sequential recruitment of motor units according to the ‘size principle’ (for discussion, see Sargeant and Jones, 1995). In contrast, in the present experiments at 39%, and even at 72% of the maximal available force, we found no evidence of type IIAX fibre recruitment. In another study by Vøllestad and colleagues (1984), subjects cycled till exhaustion at 75% VO_2max which exercise intensity would have required ~40% of the maximum available dynamic force at that velocity of contraction. They concluded that both type I and IIA fibres, which together comprised ~80% of the total fibre population, had been activated after the first bout of 20 min. The present data are consistent with these earlier observations, as at 39% MVC both type I and IIA fibres show a shift in the cumulative distribution and thus were activated. However, with increasing exercise time (~50 min), Vøllestad et al. (1984) found a decline in the PAS stain of both IIAB and IIB fibres. This would indicate a sequential pattern of recruitment associated with duration of exercise and fatigue processes including metabolic depletion, which may obscure the initial pattern of recruitment in the fresh muscle. In another study, Andersen and Sjøgaard (1976) concluded that after 14 min cycling at 85% VO_2max, which would have been equivalent to ~58% of the maximal force, all fibres showed evidence of glycogen depletion and hence recruitment. In contrast, the present results indicate that type IIAX fibres were not active at isometric contractions requiring even 72% of the available force in the fresh muscle performing just 7 contractions. This suggests again that the observed pattern of glycogen depletion was due to the relatively long duration of the exercise required in order to detect significant changes in the PAS staining intensity for glycogen.

Thus, when using glycogen depletion to study fibre activation it is difficult to separate the influence of exercise duration and intensity. When activation of certain fibres is detected after sustained exercise lasting for some minutes at any given intensity, these fibres could have been activated from the start of the exercise or later during the exercise to compensate for fatigue and/or metabolic depletion of glycogen in those fibres that had been initially recruited.

Rate coding

The shift in the cumulative frequency distribution for the PCr/Cr ratio of the type I fibres from rest to 39% MVC indicates the expected recruitment of type I fibres. The further shift of the distribution for type I fibres towards lower values at 72% MVC could be a result of increasing firing rates of the activated type I fibres and possibly an element of hierarchical recruitment within the type I fibre population. However, after the exercise involving 7 contractions at 87% MVC there is a further shift to lower values for PCr/Cr ratios, with a decrease of the mean PCr/Cr ratio of a further 29% when compared with 72% MVC. On a strictly hierarchical recruitment order the possible contribution of type I fibres to force would be ~50% of maximum and so evidence of increased energy turnover (decrease in PCr) beyond this intensity would suggest an element of rate coding.

The significant shift in the cumulative distribution for PCr/Cr for type IIA fibres at 39% MVC and a reduction of 11% in the mean value (Table 1) compared with rest also points to a significant element of rate coding since type I fibres alone might be expected to be able to deliver 39% MVC if they had been fully activated. Again in the type IIA fibres there is a further shift towards lower PCr/Cr values at 72% and a further shift at 87% MVC. That latter shift could also be interpreted as indicating an element of rate coding and increased metabolic turnover since 72% of the available force might be estimated to exceed, or be close to, the maximum force generating capability of the combined type I and type IIA fibre populations when fully activated.

It could be hypothesised that there is strategic pattern of recruitment, which matches the firing frequency in relation to the fatigability of the fibre population such that the relative metabolic stress might be similar for the type, I and the type IIA. Adopting such a strategy at submaximal exercise intensities would preserve the functional capability of the whole muscle in contrast to the selective metabolic depletion of the highest hierarchy fibre populations which occurs in maximal exercise involving 10 seconds (approximately 20 contractions) where fatigue appears to be related to high energy phosphate depletion in the most fatigue sensitive fibre populations (Karatzaferi et al., 2001b).

Advantages and limitations of the new method

The experimental method used in the present study consists of dissecting single fibre fragments from freeze-dried needle biopsies. The fragments are split into two parts, one for fibre type characterization and one for analysis of PCr and Cr content. The technique is very delicate and somewhat laborious. Nevertheless 900 fibre fragments were isolated, characterized and analysed for metabolites (the total number of fibres of each muscle biopsy was between 20 and 69). Despite its time consuming nature, the unique value of the present technique is that it provides information about fibre activation after very few contractions and hence the results are likely to be relatively uncontaminated by the effect of duration, sequential fatigue and metabolic depletion.

This is in contrast to the glycogen depletion method for which exercise of sufficient duration is needed to detect a decline in the PAS staining intensity.

It should be noted that most of the glycogen depletion studies have investigated fibre type recruitment during dynamic exercise. To our knowledge, there is only one study investigating fibre activation patterns in isometric contractions using glycogen depletion (Gollnick et al., 1974a). In that study, repeated contractions were performed till exhaustion at exercise intensity ranging from 10 to 50% MVC. At tensions less than 20% MVC glycogen was mainly depleted in type I fibres. After exercise at more than 20% MVC for at least 6 min, type II fibres showed a depletion of glycogen but not the type I fibres. This latter finding is in contrast to the present results which show that type I fibres are active at all the investigated intensities. Gollnick et al. (1974a) suggested that this apparently surprising finding was related to the lower anaerobic glycolytic capacity of type I fibres. During isometric exercise the blood flow is restricted and glycogen degradation is mainly anaerobic. As the anaerobic capacity of type I fibres is ~50% of the type II fibres (Essen et al., 1975), it can be expected that glycogen depletion would mainly occur in type II fibres. Thus it is difficult to draw clear conclusions with respect to fibre type recruitment patterns using glycogen depletion as a marker for metabolic activity in isometric exercise (Gollnick et al., 1974a). The fact that the rate of PCr breakdown, as measured in the present study, is not dependent on oxygen availability is an advantage of this new method.

Any resynthesis during or after the contractions influences the sensitivity of the method (Beltman et al., 2004a). Biopsies were obtained and frozen as quickly as possible after the end of exercise (average ~5½ s) to maintain the sensitivity of the method. Differences in sampling time, however, can cause some variability in the PCr/Cr ratio between subjects and it must be noted that this is a difficulty using the present method.

Due to the rather time consuming nature of the present technique, a limited number of subjects could be included in the study. Unfortunately, some biopsies had to be excluded from the study as a result of which some subjects had missing data. To be able to perform statistical analysis, data of the subjects was pooled for the different fibre types. Variance between subjects cannot be deduced using this analysis. In our opinion, the most reasonable statistical analysis was to show cumulative distributions of the PCr/Cr ratio and subsequently performing

Kolmogorov-Smirnov one-sided two-sample tests. It could be suggested that methodological restrictions limit firm conclusions to be made. Nevertheless, the well-known size principle could be shown using this new method. Moreover, the present method has several advantages over the glycogen depletion method as described above.

Conclusion

In the present study, a new method to assess fibre activation in short-term exercise has been applied to investigate the activation of fibre populations after 7 voluntary isometric contractions at different intensities. The strength of this method is that activation patterns of type I, IIA and IIAX fibre populations can be investigated after very brief exercise. The effect of sequential activation of fibres as a result of exercise duration, as in the glycogen depletion method, is minimised. The results indicate an hierarchical recruitment of the fibre type populations in isometric contractions performed by human muscle, but with evidence of some degree of rate coding for the type I and IIA fibre populations. In contrast to other studies, there was no evidence for recruitment of the type IIAX fibre population even at 72% of the maximum available force. It is interesting to compare this with dynamic exercise where VO_2max might be typically achieved using ~50% of the maximum available force and at which force level all fibre types have been reported to be active on the basis of glycogen depletion. In the present study it was only at 87% MVC that evidence of recruitment of the IIAX fibre population was found.

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CHAPTER 5

Metabolic cost of lengthening, isometric and shortening contractions in maximally activated rat skeletal muscle

ABSTRACT

Aim: The present study investigated the energy cost of lengthening, isometric and shortening contractions using a rat model (n=19). **Methods:** With electrical stimulation the rat medial gastrocnemius muscle was maximally activated to perform 10 lengthening, isometric and shortening contractions (velocity 25 $mm \cdot s^{-1}$) under experimental conditions (e.g. temperature, movement velocity) that resemble conditions in human movement. **Results:** Mean ± SD force-time-integrals of the first contractions were significantly different between the different protocols, 2.4 ± 0.2, 1.7 ± 0.2 and 1.0 ± 0.2 Ns, respectively ($P<0.05$). High-energy phosphate consumption was not significantly different between the three modes of exercise but a trend could be observed from lengthening (7.7 ± 2.7 $\mu mol \sim P \cdot muscle^{-1}$) to isometric (8.9 ± 2.2 $\mu mol \sim P \cdot muscle^{-1}$) to shortening contractions (10.4 ± 1.6 $\mu mol \sim P \cdot muscle^{-1}$). The ratio of high-energy phosphate consumption to force-time-integral was significantly lower for lengthening (0.3 ± 0.1 $\mu mol \sim P \cdot Ns^{-1}$) and isometric (0.6 ± 0.2 $\mu mol \sim P \cdot Ns^{-1}$) contractions compared with shortening (1.2 ± 0.2 $\mu mol \sim P \cdot Ns^{-1}$) contractions ($P<0.05$). **Conclusion:** The present results of maximally activated muscles are comparable with data in the literature for voluntary human lengthening exercise showing that the energy cost of force production during lengthening exercise is ~30% of that in shortening exercise. The present study suggests that that this finding in humans probably does reflect intrinsic muscle properties rather than effects of differential recruitment and/or coactivation.

INTRODUCTION

During exercise skeletal muscles can be active in three different modes, i.e. while being stretched, at constant muscle length or while shortening. In daily life, these three modes of contractions (lengthening, isometric and shortening) are used in combination during many movements, including locomotion.

The energy cost, measured as oxygen consumption in humans, has been shown to be lower for lengthening than shortening contractions when performing at similar work levels (Abbott et al., 1952; Bigland-Ritchie and Woods, 1976; Sargeant and Dolan, 1987). Moreover, lower EMG activity was found for lengthening compared with shortening work (Bigland-Ritchie and Woods, 1976; Westing et al., 1991). These data suggest that, to produce the same amount of force, fewer fibres are active during lengthening exercise and/or fibres are active at a lower activation level compared with shortening exercise (Abbott et al., 1952). The group of Nardone (1989) has suggested that there may be a selective recruitment of type II fibres in lengthening contractions, that is, a reversal of the normal hierarchy of motor unit recruitment according to the 'size principle'. In addition, coactivation of antagonist muscles could affect the energy cost of voluntary exercise such that the higher the antagonist activity the higher the measured total energy cost. Coactivation levels have been reported to range from ~10 to 55% of the EMG activity of the hamstring muscle when working as an agonist (Beltman et al., 2003; Grabiner et al., 1989; Kellis and Baltzopoulos, 1998; Osternig et al., 1995).

On the other hand, studies on isolated fibres or fibre bundles have shown that the energy requirement is lower for lengthening contractions than for isometric and shortening contractions (Curtin and Davies, 1973; Wilkie, 1968). Thus, both intrinsic differences in the contractile apparatus, coactivation and/or different activation of muscle fibres could result in lower metabolic cost of lengthening exercise.

Most studies using isolated muscle or fibre (bundles) measured heat during a brief period of shortening or lengthening (Barclay et al., 1993; Curtin, 1990; Linari et al., 2003). In whole body human exercise, movement usually starts as soon as muscles are activated (Sargeant and Davies, 1977): as a result shortening of muscle fibres may be faster and lengthening of fibres may be slower than in the isolated muscle because of length changes in series elastic structures. Moreover, the difference in energy cost between modes of contraction could be expected to be relatively smaller when taking into account the relatively high energy cost related to force development and relaxation. Hence, these uncertainties make the magnitude of the influence of shortening or lengthening on overall energy utilisation in muscles during human movement relatively uncertain.

Therefore, we performed experiments in which an attempt was made to simulate muscle contractions occurring *in vivo,* but with electrical stimulation of *in situ* rat muscle at physiological temperatures. Relatively low lengthening and shortening velocities were chosen to approximate to those occurring in many human locomotory movements. For example, cycling at a rate of 60 rpm is approximately the optimal cycling rate for power output for type I fibres and ~25% of the mean optimal rate for the type II fibres (Sargeant and Jones, 1995). The present method allowed us to specifically explore the energy consumption during maximally stimulated contractions where differential recruitment or coactivation of antagonist muscles is eliminated.

The purpose of the present study was to compare the metabolic cost of maximally activated skeletal muscle during short-term lengthening, isometric and shortening exercise at velocities that are comparable with human movements *in vivo*. We hypothesised that high-energy phosphate consumption would be lower during lengthening and higher during shortening contractions compared with isometric contractions.

METHODS

Muscle preparation

All experiments conformed to the 'European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes' (Council of Europe No. 123, Strasbourg 1985), and were approved by the Ethical Committee for Animal Research of the Vrije Universiteit. Experiments were performed using male Wistar rats (n = 19, body mass 281 ± 19 g) anaesthetised with urethane (1.5 $g \cdot kg^{-1}$ body mass intraperitoneally). Supplementary injections of 0.63 $g \cdot kg^{-1}$ body mass intraperitoneally were given if necessary. The experimental procedures have been described previously (Abbate et al., 2000; de Haan et al., 1989) and are summarised below. The medial gastrocnemius muscle-tendon complex was prepared free from the surrounding tissue without compromising the blood supply. The animal was placed prone on a heated pad (35°C) to maintain body temperature with the femur of the operated leg clamped in a vertical position and the muscle held horizontally. The tendon was connected to a force transducer, which was part of an isovelocity measuring system (de Haan et al., 1989). Stimulation was performed through the severed sciatic nerve with only the branch to the medial gastrocnemius left intact. The current of each stimulation pulse was set on 1 mA, which was ~30% higher than needed for maximal force development. Pulse duration was 0.05 ms. Motor movements and stimulation were computer controlled. Force and length data were digitised (1000 Hz) and stored on disk for later analyses. At the end of the experiments, anaesthetised rats were humanely killed by an overdose of anaesthetic.

Muscle optimum length

Tetanus optimum length (L_0) was first estimated using twitches. Subsequently, L_0 was determined by using 158 ms tetani of 200 Hz. After L_0 had been established, the overall length of the muscle and most distal fibre bundle length were measured with a pair of compasses and estimated to be 32 ± 2 mm and 17 ± 2 mm (mean ± SD), respectively. Then the muscle was allowed a rest period of 15 min before beginning the experiments. With this methodology, the assessment of L_0 did not compromise energy metabolism prior to the experimental exercise (Abbate et al., 2001). Muscle temperature was controlled by a water-saturated airflow around the muscle of ~35°C. Using this technique a previous study has shown that muscle temperature was within 1°C of the temperature of the airflow (de Haan, 1998).

Contraction protocols

Muscles were randomly assigned to either the control (rest) condition (n = 7) or the lengthening (n = 7), isometric (n = 7) or shortening (n = 7) protocol. The three imposed protocols consisted of a series of either 10 lengthening, isometric or shortening contractions (stimulation frequency 200 Hz, stimulation duration 158 ms, cycle duration 500 ms). The series of isometric contractions was performed at L_0. For the lengthening contractions the motor performed a movement ($v = 25\ mm \cdot s^{-1}$) from L_0-4 mm to L_0, for the shortening contractions this movement ($v = 25\ mm \cdot s^{-1}$) was from L_0 to L_0-4 mm. Stimulation and motor movement stopped simultaneously and relaxation occurred after the movement, at the end length. Using this set-up, muscles were active over the same trajectory in lengthening and shortening contractions. Just before the start of the series of contractions, the blood flow to the medial gastrocnemius muscle was occluded to minimise aerobic metabolism and to prevent removal of lactate from the muscle. Then, the series of 10 repeated contractions was started, after which the muscle was quickly (within 2 s) freeze-clamped with a pair of tongs precooled in liquid nitrogen. Subsequently, the muscle was excised and stored in liquid nitrogen until further analysis. For the control muscles, the same procedure was followed except for the series of contractions.

Figure 1 shows examples of the force signals of a lengthening, isometric and shortening contraction. Force-time-integral (FTI) was calculated for each contraction by integrating the force over time.

Choice of movement and velocity

In a recent study from our group (de Haan et al., 2003) it has been shown that, in shortening contractions, muscles produce their maximal power output not at optimum length but at lower length, L_0-2 mm. Therefore, we have set the range of movement between L_0 and L_0-4 mm.

The purpose of the present study was to investigate metabolic costs of the three modes of contractions at movement velocities that are comparable with *in vivo* movements like running and cycling. Therefore, we have chosen a relatively low movement velocity (25 $mm \cdot s^{-1}$), which is at ~1/3 of the optimum velocity for power output of rat medial gastrocnemius muscle (de Haan, 1998) and is comparable with human cycling exercise at relative low cycling frequencies (60 rpm).

Analysis of metabolites

The frozen muscles were ground in a mortar under constant addition of liquid nitrogen. The muscle powder was then freeze-dried overnight and stored in liquid nitrogen until further analysis. Metabolites were separated and quantified using a high-performance liquid chromatography (HPLC) system that consisted of a Binary LC pump (Model 250, Perkin-Elmer, USA), an autosampler with cooling tray and automatic injector (Basic Marathon, Spark Holland, the Netherlands) and a variable-wavelength ultraviolet (UV) spectrophotometric detector (Model 759A, Applied Biosystems, the Netherlands). The size of the injection loop was 20 µl and the UV absorption was measured at λ = 254 nm, λ = 210 nm and λ = 260 nm for nucleotides, creatine compounds and lactate, respectively.

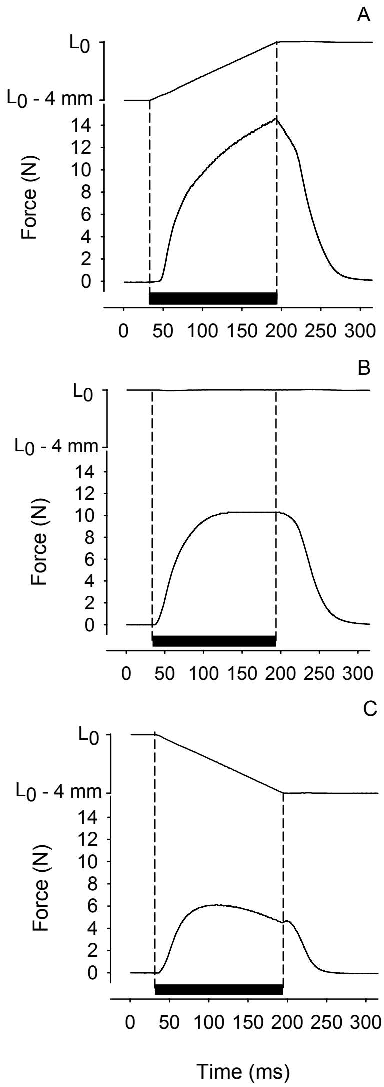

Figure 1. Example of a force signal during maximal A) lengthening, B) isometric and C) shortening contractions. Force (N) is shown in the lower trace and length (mm, relative to muscle optimum length (L_0)) is shown in the upper trace of each figure. The dotted line indicates the start and end of the movement (in A and C) and indicates also the time interval over which the force-time-integral was calculated (in A, B and C). The black horizontal bar represents the stimulation period.

Analysis of ATP, inosine 5-monophosphate (IMP), creatine phosphate (PCr) and creatine (Cr) was carried out as previously described (Abbate et al., 2002; Karatzaferi et al., 1999). The lactate analysis was performed according to the method of Simonides et al. (1988) and summarised below. Prior to HPLC analysis, lactate duplicate extractions were performed by homogenising >150 µg of dry muscle tissue in 50 µl perchloric acid (0.6 M). The muscle tissue was sonicated and briefly centrifuged (16000 g, Centrifuge 5415 from Eppendorf), after which 40 µl of the supernatant was neutralised to pH 6.5-7.0 with 5.5 µl K_2CO_3/Tris solution (2.8M K_2CO_3, 0.1 M Tris). The neutralised homogenate was centrifuged for 20 minutes (25000 g, 4°C, Biofuge 22R from Heraeus Sepatech) and 20 µl KOH (50mM) was added to 40 µl of the supernatant. Then, the supernatant was freeze-dried and stored at –80°C for further analysis. For derivatisation of lactate, 250 µl acetonitrile (containing 1.67 mM di-bromo-acetophenone and 83 µM 18-crown-6 ether) was added, sonicated for 5 min and gently shaken for 20 min at 80°C. The sample was subsequently cooled on ice and centrifuged. For HPLC analysis 120 µl of the supernatant was used. Separation occurred at controlled room temperature (20°C) under isocratic condition using a Lichrosorb RP8 (10 µm) column (40 x 250 mm, Merck). The flow rate was 3 ml·min^{-1} using a mobile phase of acetonitrile and H_2O (27:73) for 8 min. Then, acetonitrile was raised to 50% for 5 min and lowered again to 27:73 for 5 min.

High-energy phosphate consumption

The concentrations of ATP, IMP, PCr and Cr in each muscle were normalised for the average total amount of creatine (PCr + Cr = 117.7 ± 19.9 µmol·g^{-1} dw) to correct for errors in weighing of the muscle samples. High-energy phosphate consumption (HEPC) was calculated from the differences in metabolite concentrations between the experimental and the average of the resting muscles using the following formula:

$$HEPC = 1.5\Delta[lactate] - \Delta[PCr] - \Delta[ATP] + \Delta[IMP]$$ (Westra et al., 1982).

HEPC was then multiplied by 0.23 (dry·wet^{-1} mass ratio) (de Haan et al., 1986) and by average muscle mass (794 ± 70 mg) to obtain HEPC per muscle (µmol~P·muscle^{-1}). Due to the quick freeze-clamp procedure, there was a relatively large variation in measured muscle mass that was not related to body mass.

Therefore, we decided to multiply by average muscle mass instead of the individual muscle mass. To compare the energy cost for mechanical output of the present study with data of oxygen consumption in human voluntary exercise we expressed the high-energy phosphate consumption per unit FTI (HEPC·FTI^{-1} in µmol~P·Ns^{-1}).

Statistical analysis

One-way analyses of variance (ANOVA) were used to test for differences in FTI, metabolites and high-energy phosphate consumption between the four groups. To test for significant differences between the group means, post-hoc comparisons were made using the Bonferroni method. The level of significance was set at $P<0.05$. Results are presented as mean ± SD.

RESULTS

Force and force-time-integral

The mean peak force of the first contractions of the lengthening, isometric and shortening protocol was respectively, 16.3 ± 1.4, 10.1 ± 0.9 and 6.8 ± 1.0 N, all being significantly different from the other ($P<0.05$). The same is true for the mean FTI of the first contractions, which values were 2.4 ± 0.2, 1.7 ± 0.2 and 1.0 ± 0.2 Ns, respectively ($P<0.05$). There was a progressive moderate fatigue during the exercise in all modes (Figure 2). The decline in FTI of the isometric contractions (10.1 ± 7.3 %) was significantly smaller ($P<0.05$) than of the lengthening (20.7 ± 3.5 %) and shortening (21.5 ± 6.5 %) contractions. The total FTI for the lengthening contractions (23.0 ± 2.6 Ns) was significantly higher than for the isometric (16.2 ± 2.3 Ns) and shortening (9.1 ± 1.7 Ns) contractions ($P<0.05$) (Figure 3A). In addition, total FTI values for the isometric protocol were significantly higher than for the shortening protocol ($P<0.05$).

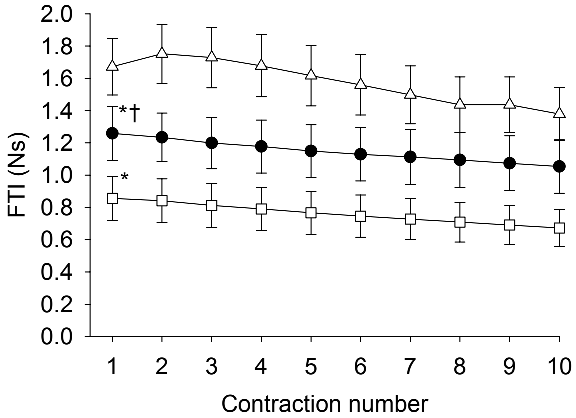

Figure 2. Mean ± SD force-time-integral (FTI) for the series of 10 maximal lengthening (Δ), isometric (•) and shortening (□) contractions. FTI of the first contraction was significantly different from *lengthening and †shortening contractions (P<0.05).

High-energy phosphate consumption

The concentrations of the high-energy phosphates and lactate at rest and after the series of 10 lengthening, isometric and shortening contractions are shown in Table 1. No significant differences were found in ATP and IMP levels between rest and post exercise conditions. PCr, Cr and PCr/Cr values after each series of 10 contractions were significantly different from the resting values (P<0.05). Lengthening post-exercise PCr and Cr concentrations were significantly different from the shortening protocol (P<0.05) as was the PCr/Cr ratio (P<0.05). Lactate levels after the three contractions protocols were significantly different from values at rest (P<0.05). No significant differences were detected between the contraction protocols.

<u>HEPC</u>

HEPC was calculated for each protocol from the difference in the metabolite concentrations pre- and post-exercise. Mean HEPC for the lengthening, isometric and shortening contractions were 7.7 ± 2.7, 8.9 ± 2.2 and 10.4 ± 1.6 μmol~P·muscle^{-1}, respectively. There was a trend for an increase in mean HEPC from lengthening to isometric to shortening contractions (Figure 3B), however no significant differences were observed.

Table 1. Concentrations of metabolites at rest and after 10 repeated maximal lengthening, isometric and shortening contractions.

	PCr	Cr	PCr/Cr	ATP	IMP	Lactate
Rest	81.5 ± 5.0	36.3 ± 5.0	2.30 ± 0.45	23.5 ± 1.8	0.28 ± 0.28	9.2 ± 2.8
Lengthening	56.2 ± 5.6*†	61.5 ± 5.6*†	0.93 ± 0.17*†	22.8 ± 3.0	0.74 ± 0.79	19.6 ± 7.2*
Isometric	50.7 ± 6.0*	67.0 ± 6.1*	0.77 ± 0.16*	23.6 ± 0.8	0.60 ± 0.16	21.1 ± 5.1*
Shortening	46.0 ± 3.2*	71.8 ± 3.2*	0.64 ± 0.08*	23.1 ± 2.1	1.04 ± 0.58	22.8 ± 4.8*

Values are mean ± SD in μmol·g dw^{-1}. PCr, phosphocreatine; Cr, creatine; PCr/Cr, ratio of PCr to Cr; IMP, inosine 5-monophosphate. Significantly different from *rest and †shortening contractions ($P<0.05$).

HEPC/FTI

The energy cost for mechanical output (HEPC/FTI) was significantly lower for the lengthening (0.3 ± 0.1 μmol~P·Ns^{-1}) and isometric (0.6 ± 0.2 μmol~P·Ns^{-1}) contractions compared with shortening contractions (1.2 ± 0.2 μmol~P·Ns^{-1}, $P<0.05$) (Figure 3C). The difference in HEPC/FTI between lengthening and isometric contractions did not reach significance ($P=0.059$).

DISCUSSION

The main finding of the present study was that the energy cost for mechanical output (HEPC/FTI) of a muscle *in situ* was different between maximal lengthening, isometric and shortening contractions when assessed under circumstances that are comparable with human movement. The lowest HEPC/FTI was observed for the lengthening protocol. We were unable to demonstrate significant differences in metabolic cost (HEPC) between the three modes of contractions, despite the fact that there were the significant differences in FTI between those protocols.

High-energy phosphate consumption

The results of the present study show that the ratio of HEPC/FTI was lowest for lengthening contractions of the muscle tendon complex (Figure 3C). This was expected as it is known that when a muscle is shortened the ATP usage increases (Barclay et al., 1993; Kushmerick and Davies, 1969) whereas when a muscle is lengthened the ATP usage decreases (Curtin and Davies, 1973). The cross-bridge model can explain this. In lengthening contractions, the muscle is stretched and

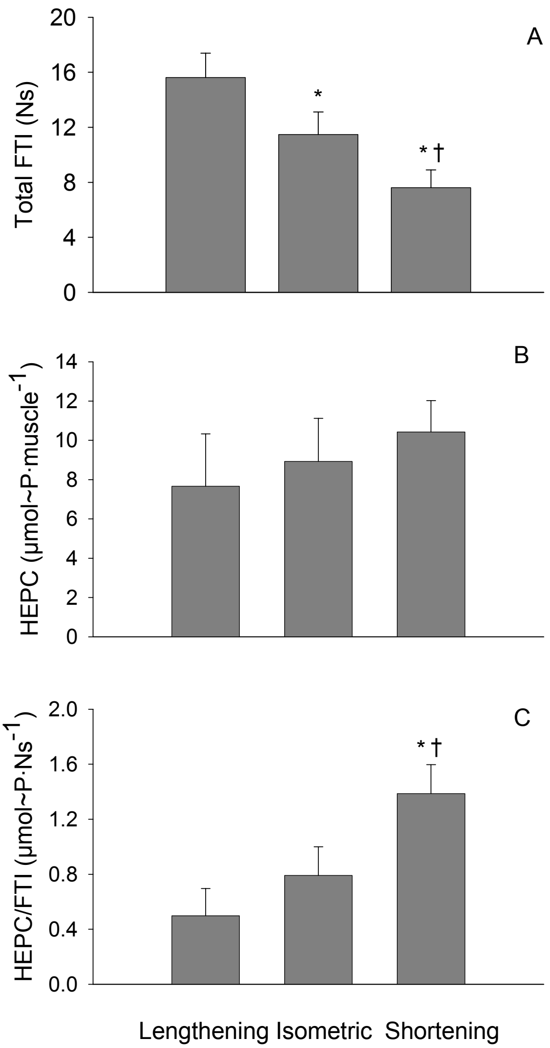

Figure 3. Mean ± SD A) total force-time-integral (total FTI), B) high-energy phosphate consumption (HEPC) and C) ratio of HEPC to FTI for/after the series of 10 maximal lengthening, isometric and shortening contractions. Significantly different from *lengthening ($P<0.05$) and †isometric contractions ($P<0.05$).

activated cross-bridges are pulled apart. As a result of this stretch, higher forces are produced compared to isometric and shortening contractions and this increase in force is dependent on the velocity of the stretch. According to the Huxley model (1957), ATP hydrolysis is necessary for detachment. However, in the case of forced lengthening during contraction, there will be cross-bridges in which the binding between actin and myosin is mechanically broken rather than by ATP hydrolysis (Flitney and Hirst, 1978).

The metabolic cost of lengthening contractions in maximally activated muscles was not significantly different from isometric and shortening contractions (Figure 3B). This may be related to the relatively large variation in HEPC values (SD of 15-35%) but can also be a result of the relatively low velocity used in the present experiment such that many cross-bridges are detaching with ATP hydrolysis. Moreover, the absolute difference between the mean HEPC of lengthening and shortening contractions is relatively small, only 2.8 μmol~P·muscle^{-1} which is 26%. Nevertheless, a trend could be observed for an increasing energy cost going from lengthening to isometric to shortening contractions.

In situ versus in vivo exercise

Several studies have determined the metabolic cost of different types of contractions in humans. The general conclusion was that there are large differences in metabolic strain between the three modes of exercise at submaximal work levels with the lowest cost for lengthening contractions. The oxygen consumption for lengthening exercise has been shown to be ~35% (Abbott et al., 1952; Bonde-Petersen et al., 1972) of that for shortening exercise for cycling and ~20-40% (Margaria et al., 1963) for walking and running. To compare the present results with those experiments, which are performed at comparable workloads for lengthening and shortening contractions, the present study has expressed the energy cost per unit FTI (HEPC/FTI). The HEPC/FTI for lengthening contractions was 63% and 36% of the energy cost for isometric and shortening contractions, respectively. Thus, the difference in energy cost between lengthening and shortening contractions in maximally activated muscles is comparable with observations in human voluntary exercise. This would suggest that different activation patterns or coactivation in voluntary exercise do not have a major impact on the energy consumption but that the determining factor for energy requirement is located in the muscle, possibly at cross-bridge level.

Experimental model

In the present experiment, the energy cost of three modes of contractions has been determined using only 10 electrically stimulated contractions in a rat model. The advantage of such a model over using single fibres or (muscle) bundles is that the interaction in a muscle-tendon complex can be studied under more physiological conditions, which resemble those in human movement. This model has also several advantages over human studies. First, the energy requirement can be investigated for muscles of which all fibres are maximally activated and thus the possible influence of different activation patterns and coactivation is eliminated. Second, as only a few contractions were performed there was only a moderate effect of fatigue (~15-20%), which would have a relatively small effect on the total energy cost. The observation that there is no change in ATP and IMP concentrations after the short-term high intensity exercise also indicates that the effect of fatigue can be neglected (de Haan et al., 1993; de Haan and Koudijs, 1994). Third, only a relatively small stretch has been used (4 mm) which probably has caused a stretch of the muscle fibres of only ~2-2.5 mm (Ettema et al., 1990). The remaining of the length change is absorbed by the series elastic component of the muscle-tendon complex. As a consequence, there would have been hardly any damage in the muscle during the lengthening contractions as occurs in prolonged human exercise (Clarkson and Tremblay, 1988; Jones et al., 1986; Sargeant and Dolan, 1987). So, with the method used in the present experiment the energy cost of maximal lengthening, isometric and shortening contractions could be determined for a muscle tendon complex under physiological conditions without disturbing influences of activation, antagonist coactivation, fatigue or damage.

Force-time-integral and metabolic parameters

As would be expected from the *in vitro* force-velocity relationship (Edman et al., 1978; Katz, 1939), the present study showed that force and FTI of lengthening contractions were higher than of isometric and shortening contractions. In addition, the force and FTI during isometric were higher than in shortening contractions. The lengthening-to-isometric ratio of peak force was 1.6 and for FTI this value was 1.4. Comparable peak force ratios (1.2-1.4) were found in *in vivo* contractions using electrical stimulation to maximally activate muscle (de Ruiter et al., 2000; Westing et al., 1990).

In contrast to the force and force-time integral, which was highest in lengthening contractions, we could not demonstrate significant differences in metabolites after maximally activated lengthening, isometric and shortening contractions. Significant differences were only found in the creatine compounds after lengthening exercise (Table 1). PCr decrease and Cr increase were significantly smaller (29%) compared with shortening exercise. Consequently, the decline in PCr/Cr ratio was significantly smaller after lengthening contractions (17%). The decline in PCr/Cr ratio after isometric exercise (~70%) is comparable with values found after 7-10 maximal voluntary isometric contractions with the human quadriceps (Beltman et al., 2004a).

Conclusion

In the present study the energy cost for mechanical output was investigated for a muscle-tendon complex operating under circumstances simulating human movement conditions. However, in contrast to human voluntary movement the muscle was maximally activated using electrical stimulation. The results show that, when muscles are maximally activated, the energy cost per unit FTI is lowest for lengthening contractions. Data in the human literature also indicate that *voluntary* lengthening exercise has a similar lower energy requirement compared with isometric and shortening exercise usually based on pulmonary oxygen uptake. The present data confirm the validity of this approach to studying the energy cost of human movement. Furthermore the results indicate that the often suggested role of differential recruitment or coactivation in human voluntary exercise is probably not a major factor determining the energy cost of movement involving different types of contraction. Hence the difference in energy cost between the three modes of contractions at relatively low velocities is suggested to be an intrinsic property of the muscle, presumably located at the cross-bridge level.

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CHAPTER 6

Lower voluntary activation of human quadriceps muscle during lengthening contractions: no evidence for selective recruitment of type II fibres

ABSTRACT

Voluntary activation level during lengthening, isometric and shortening contractions was investigated using electrical stimulation of the femoral nerve (triplet, 300 Hz) superimposed on maximal efforts. Recruitment of fibre populations was investigated using the PCr/Cr ratio of single characterized muscle fibres obtained from needle biopsies at rest and immediately after series of 10 lengthening, isometric and shortening contractions (1 s on/1 s off). Maximal voluntary torque was significantly higher during lengthening (270 ± 55 Nm) compared with shortening contractions (199 ± 47 Nm, P<0.05) but not different from isometric contractions (252 ± 47 Nm). Isometric torque was higher than torque during shortening (P<0.05). The estimated maximal torque generating capacity was significantly different (P<0.05) between lengthening (342 ± 68 Nm), isometric (273 ± 54 Nm) and shortening (213 ± 53Nm) contractions, as was the triplet torque evoked in resting muscle (136 ± 36, 105 ± 22 and 82 ± 18 Nm, respectively). Voluntary activation level during lengthening contractions (79 ± 8%) was significantly lower compared to isometric (93 ± 5%) and shortening contractions (92 ± 3%, P<0.05). Mean PCr/Cr ratios at rest were 2.5 ± 0.6, 2.0 ± 0.7 and 2.0 ± 0.7 for type I, IIA and IIAX fibres, respectively. After 10 contractions the mean PCr/Cr ratios for grouped fibre populations were significantly different from rest, 1.3 ± 0.2, 0.7 ± 0.3, 0.8 ± 0.6 for lengthening, isometric and shortening contractions, respectively (P<0.05). The cumulative distributions of individual fibre populations were significantly different from rest (P<0.05). Curves after lengthening contractions were less shifted compared to curves from isometric and shortening contractions (P<0.05). The results indicate a reduced voluntary drive during lengthening contractions. PCr/Cr ratios of single fibres could not provide evidence for selective recruitment of type II fibres.

INTRODUCTION

From the literature on animal whole muscle preparations or single fibres it is well known that force during lengthening contractions increases above the isometric force (Edman et al., 1978; Katz, 1939). In human voluntary lengthening contractions, however, this higher torque could, in general, not be demonstrated (Babault et al., 2001; Dudley et al., 1990; Westing et al., 1988; Westing et al., 1990). This divergence from the *in vitro* relationship is often attributed to a tension limiting mechanism, which limits motor unit recruitment and/or discharge rate during lengthening contractions (Webber and Kriellaars, 1997; Westing et al., 1990).

Studies using surface electromyography (EMG) activity during maximal isometric and isokinetic exercise have shown that EMG levels during lengthening contractions are lower than during shortening contractions though higher force output is obtained (Aagaard et al., 2000b; Kellis and Baltzopoulos, 1998; Komi et al., 2000; Westing et al., 1991). Using superimposed electrical stimulation, it has been demonstrated that the torque of voluntary lengthening contractions can be increased (Amiridis et al., 1996; Dudley et al., 1990; Westing et al., 1990). In addition, by means of the twitch interpolation technique, activation levels during maximal voluntary lengthening contractions have been shown to be lower than during isometric contractions (Babault et al., 2001). These results from studies using different methods support the notion of a reduced neural drive during lengthening contractions compared to isometric and shortening contractions. It has been suggested that the reduction in neural drive could be due to either a lower activation of all recruited fibres as a consequence of inhibition, or due to activation of selective fibre populations (and inhibition/de-recruitment of other fibre populations) during lengthening contractions (Enoka, 1996). This would lead to a reversal of the normal hierarchy of recruitment (Howell et al., 1995; Linnamo et al., 2003; Nardone et al., 1989; but cf. Kossev and Christova, 1998; Søgaard et al., 1996).

In the present investigation we have examined the level of activation during maximal voluntary lengthening, isometric and shortening contractions and related this to evidence for activation of different fibre type populations. For this purpose, the ratio of phosphocreatine to creatine (PCr/Cr) was measured in single characterized fibre fragments, which were isolated from needle biopsies obtained at rest and after only 10 lengthening, isometric and shortening contractions. In an earlier study (Beltman et al., 2004a) we have demonstrated that this ratio is a useful indicator of fibre activation after very brief exercise involving only a few contractions of 1 s duration. This method enabled us to assess whether type II fibres had been selectively activated (associated with a de-recruitment or inhibition of type I fibres) or whether all fibres were activated at a lower level during lengthening contractions.

Thus the first aim was to investigate the voluntary activation level during lengthening, isometric and shortening contractions using the superimposed stimulation technique. For this purpose, high frequency triplets were applied to the femoral nerve during maximal voluntary knee extension contractions and on the relaxed muscle. The second aim was to investigate the activation of different fibre populations during these modes of contraction using the PCr/Cr ratio in single fibres. We hypothesised that we would find lower activation levels of lengthening contractions compared to isometric and shortening contractions as evidenced by superimposed stimulation, and that this would be associated with a lower decrease in the PCr/Cr ratio in all fibre types indicating a maintenance of the normal hierarchy of fibre type recruitment rather than any reversal of recruitment pattern.

METHODS

Two experiments were performed with the approval of the ethical committee of the Vrije Universiteit Medical Centre, Amsterdam, The Netherlands and in accordance with the Declaration of Helsinki. After the procedures of the experiment were explained, all subjects gave oral and written informed consent.

Study design

In Experiment 1, the voluntary activation level was determined during lengthening, isometric and shortening contractions using superimposed electrical stimulation elicited on maximal efforts. Subjects came to the laboratory for two sessions. In the first session the torque-knee angle relationship was determined for each subject. In addition, voluntary activation level during either lengthening, isometric or shortening contractions was tested. In the second session, which was at least two days after the first, the voluntary activation level of the remaining two contraction modes was determined. The order in which the lengthening, isometric and shortening protocols were carried out was randomised over the two sessions.

In Experiment 2, five subjects came to the laboratory for a session in which needle biopsies were obtained after three separate series of 10 contractions performed under lengthening, isometric and shortening conditions.

Torque measurements

Isometric and isokinetic knee extension exercise was performed on a specially designed dynamometer, which allowed torque measurements at preset angular velocities. Subjects sat in an upright position with a hip angle of ~75° (0° = full extension) and the axis of rotation of the dynamometer was aligned with the lateral femoral condyle. Straps were applied to the pelvis and torso to stabilise the subject. For all subjects of Experiment 1 the right leg was used for the exercise. A cuff was fastened around the lower leg and this cuff was subsequently attached to the lever of the dynamometer. Torque of the contractions was AD-converted (1000 Hz) and stored on disk for off-line analysis. All recorded torque values were corrected for the effect of gravity. To prevent fatigue a minimal rest of 2 minutes was allowed between all maximal efforts.

Experiment 1

Purpose

The aim of the first experiment was to investigate voluntary activation levels of the quadriceps muscle during lengthening, isometric and shortening contractions using the superimposed stimulation technique.

Subjects

Ten healthy subjects, six men and four women with a mean ± SD age of 28 ± 8 yr, height 179 ± 12 cm and mass 74 ± 9 kg participated in this experiment. All were regularly active with a mean of 6 training hours a week.

Electrical stimulation

To determine the voluntary activation level of the quadriceps muscle, a superimposed nerve stimulation technique was used. In the present study a triplet was used instead of a twitch (e.g. Babault et al., 2001; Becker and Awiszus, 2001) as it has been shown that the variability in superimposed torque is reduced with increasing number of stimuli (Suter and Herzog, 2001). Using a twitch, superimposed torques may sometimes seem absent though this may be due to the insensitivity of the method due to the small transient extra torque rather than complete activation (Kent-Braun and Le Blanc, 1996). Consequently, this may overestimate the voluntary activation level.

Electrical stimulation was applied to the femoral nerve during maximal voluntary knee extension efforts (isometric and isokinetic) and when the muscle was relaxed. A constant current stimulator (Model DS7, Digitimer, Hertfordshire, UK) was used with self-adhesive surface electrodes (Schwa-Medico, Nieuw Leusden, Nederland) placed on the skin. The cathode (5 x 5 cm) was placed in the trigonum femorale to stimulate the nervus femoralis; the anode electrode (8 x 13 cm) was placed on the most prominent part of the m. vastus medialis. The exact location for the stimulating electrode on the nerve was determined using a ball probe electrode. Twitches of 30-50 mA were applied and a different electrode position was used for each twitch. The smallest electrode was located at the position of the probe, which gave the largest visible muscle contraction. To determine voluntary activation level during maximal attempts, three square wave pulses (triplet) of 200 µs were delivered to the muscle at a frequency of 300 Hz, using supra-maximal current. Maximal current was determined, using the triplet, by raising the current until isometric torque at optimum knee angle did not further increase. The current was then increased by a further 20 mA to ensure supramaximality. In the second session the current was determined again. The mean current used was 162 ± 26 mA and the applied triplet evoked a muscle contraction of ~40% of maximal voluntary isometric torque. To increase the reproducibility of the electrode positioning, the positions of the electrodes were marked on the skin before they were removed in the first session.

Most of the subjects had experienced electrical stimulation before. In the subjects who had not, the intensity of stimulation was gradually increased to accustom them to the sensation.

<u>Isometric exercise</u>

In the first session the torque-knee angle relationship was determined. Subjects performed maximal isometric knee extension contractions at 90, 80, 70, 60, 50, 40, and 30° knee flexion angle in a randomised order. One maximal effort lasted ~3-4 seconds. At each knee angle two attempts were performed. When the torque of these two attempts differed more than ~10%, a third attempt was performed. The maximal torque reached in these attempts was considered to be the subject's maximal isometric torque for the specific knee angle. The knee angle at which maximal torque was measured (optimal knee angle) was used for all isometric testing protocols.

Voluntary activation level was determined for isometric contractions using superimposed stimulation. During maximal isometric knee extension contractions, triplets were elicited approximately 1.5-2 seconds after the start of the contraction (for example, see Figure 1B). As subjects sometimes had difficulties to perform a maximal effort when a triplet was to be superimposed, several attempts had to be performed until the maximal voluntary torque (before the triplet) did not further increase (usually not more than 5 attempts). For the isometric contractions, the triplets evoked at resting muscle were applied at the same knee angle as the voluntary contractions.

Isokinetic exercise

Shortening and lengthening contractions were performed at an angular velocity of respectively $60^{o}\cdot s^{-1}$ and $-60^{o}\cdot s^{-1}$ with a range of motion between 90 and 30° knee flexion angle. A small preload (25 Nm) had to be overcome to start the movement of the dynamometer lever arm. As this preload was very low, there was no isometric phase before the movement. It has been shown that preload influences the torque magnitude such that with increasing preload the torque increases (de Morton and Keating, 2002). It might be hypothesised that preload could affect the amount of voluntary activation as it has been found that motor unit activation patterns are influenced by preactivation (Linnamo et al., 2003). In the present study a relatively small preload was chosen as we aimed to investigate activation patterns of type I and II fibres during three modes of contraction (Experiment 2). An isometric preactivation would have influenced the PCr/Cr ratio such that the results from the lengthening and shortening contractions could have been obscured by the changes elicited by the isometric phase. As the movement was started, the dynamometer accelerated at $1000°\cdot s^{-2}$ to the preset angular velocity. Subjects performed 2-4 maximal lengthening or shortening attempts to get accustomed to the movement and to determine the knee angle at which maximal torque was reached. Next, superimposed stimulation was applied on maximal lengthening or shortening attempts (for example, see Figure 1A and C). For each subject, superimposed triplets were triggered such that the effect of the stimulus occurred at optimal knee angle for lengthening and shortening contractions. For the triplets evoked in resting muscle, the leg was passively moved from 30 to 90º for the lengthening mode and from 90 to 30º for the shortening mode. Similar to the superimposed triplet, the triplets in resting muscle were elicited such that highest torque occurred at optimum knee angle. As in the isometric contractions, subjects performed lengthening or shortening attempts until voluntary torque did not further increase.

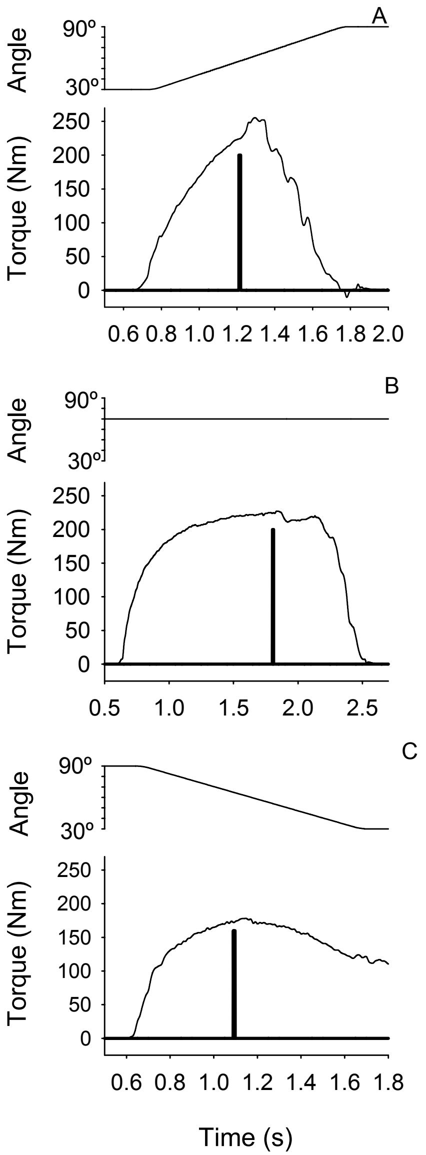

Figure 1. Example of torque signals of one subject during A) lengthening, B) isometric and C) shortening contractions. Torque (Nm) is shown in the lower trace and knee flexion angle (º) is shown in the upper trace of each figure. The thick line indicates the superimposed triplet. Note that the superimposed torque in lengthening contractions was much higher than in isometric and shortening contractions.

Torque analysis

Maximal voluntary extension torque was the highest measured voluntary torque for lengthening, isometric and shortening contractions, respectively. Torque of superimposed triplet (a) for the different contraction modes was determined by subtracting the maximal voluntary extension torque before the triplet (b) from the maximal torque evoked by the triplet. The superimposed torque was expressed relative to the torque of the triplet evoked at rest (c) for the corresponding modes. Maximal torque generating capacity (MTGC) of the muscle was then calculated using the following formula:

$$MTGC = \frac{b}{1 - a/c} \text{ (de Haan et al., 2000).}$$

Voluntary activation level was determined as the ratio between maximal voluntary torque obtained during the measurements and MTGC. This approach to assess the voluntary activation level has been chosen to allow comparison of activation levels between Experiment 1 and 2 (see “Torque analysis” of Experiment 2).

Several attempts were made at each contraction mode to perform a maximal effort. For statistical analysis we have used the attempt in which the highest voluntary torque before the triplet was reached.

Experiment 2

Purpose

The aim of the second experiment was to assess the activation pattern of type I, IIA and IIAX fibres during only 10 lengthening, isometric and shortening contractions using the PCr/Cr ratio of single characterized fibre fragments as a measure for fibre activation.

Subjects

Five healthy subjects, four men and one woman with a mean ± SD age of 30 ± 9 yr, height 181 ± 11 cm and mass 76 ± 8 kg participated in this experiment. Four of these subjects (three men and one woman) also participated in Experiment 1. All were regularly active with a mean of 4 training hours a week.

Protocol

The subjects performed three separate series of 10 maximal contractions under lengthening, isometric and shortening conditions. Each contraction was of 1 s duration (1 s rest). Each protocol was performed with either the right leg or the left leg. The order in which the series were performed and the leg which was tested was randomised, except that after lengthening exercise no other protocol was performed with that leg. Rest between series was at least 10 min. To impose the 1 s on/1 s off rhythm, an auditory signal was given during the contraction phase. Immediately after relaxation from the last contraction a needle biopsy was taken from the m. vastus lateralis. The isometric protocol was performed at optimum knee flexion angle. The preload, range of motion and angular velocity for lengthening and shortening contractions were the same as used in Experiment 1. To be able to relate the average torque attained during the contractions to the maximal torque generating capacity of the muscle, activation levels during the three contraction modes were determined for both legs. This was performed in two separate sessions (pre-sessions), which were carried out before the session in which biopsies were obtained.

<u>Muscle biopsy</u>

With the subject seated on the dynamometer and the knee in the optimum knee flexion angle, small incisions were made after local anaesthesia (2% lidocaine) of the skin and fascia. Two incisions were made (one in each leg) at one-third of the distance between the lateral femoral epicondyle and trochanter major. Immediately following the last contraction of a series, a muscle sample was collected from the m. vastus lateralis of the exercised leg using a Bergström type biopsy needle (Ø 5 mm, Popper Biomedical Instruments, Schuco International London Limited, London, UK) with suction. From each subject a resting sample was also obtained. As two biopsies were taken from one leg the needle was directed either proximally or distally. Biopsies were frozen in liquid nitrogen within an average of 3.8 s from end of exercise to freezing and they were freeze-dried overnight. The freeze-dried samples were stored desiccated in tubes of which the lid was sealed with laboratory film. Each tube was placed in another small jar with some silica gel, the lid sealed with laboratory film and stored in liquid nitrogen vapour until analysed. Individual fibre fragments of 2-3 mm length (~80 from each sample) were dissected under conditions of controlled ambient temperature and relative humidity (~20°C and <~20% RH). Each fibre fragment was divided into two parts, one for histochemistry and the remaining part for analysis of metabolites. The methods used for single fibre typing and muscle metabolite analysis are extensively described elsewhere (Karatzaferi et al., 1999; Sant'Ana Pereira et al., 1995b). Three biopsies were excluded from the study because no fibres could be dissected from the sample or because freezing time was too long (>10 s).

Histochemistry

Serial sections (~10 μm) of gelatine-embedded single fibre fragments were cut and characterized for acid mATPase stability at pH 4.6 and 4.4 (adapted from Brooke and Kaiser, 1970). An image analysis system (KS 300 Imaging System 3.0, Carl Zeiss Vision, Germany) was used to measure the optical density (OD) of histochemically treated fibre sections (Sant'Ana Pereira et al., 1995b). Based on the OD values from mATPase, pre-incubation pH 4.4, the fibres were classified into type I and II fibres. The mATPase, pre-incubation pH 4.6 was then used to divide the type II fibres into two categories, type IIA (0-25% IIX) and type IIAX (25-100% IIX)(Gerrits et al., 2003).

Analysis of metabolites

From the characterized muscle fibre fragments of each muscle biopsy, a maximum of ~20 fibres from each fibre type were analysed for phosphocreatine (PCr) and creatine (Cr) by using reverse-phase high-performance liquid chromatography (HPLC) with ultra-violet photometric detection (Karatzaferi et al., 1999) following overnight extraction in 60% methanol (Donofrio et al., 1978). The PCr/Cr ratio was used as a measure for fibre activation (Beltman et al., 2004a).

Torque analysis

For each series of contractions the peak torque of each individual contraction was determined. To determine the voluntary activation level during the series of contractions the mean of the 10 contractions for each contraction mode was expressed relative to the MTGC estimated for that leg.

Statistical analysis

All results are presented as mean ± SD. The Shapiro-Wilk test was used to check for normality of the data of Experiment 1. To test for significant differences, parametric one-way ANOVA for repeated measures was applied with contraction (lengthening, isometric and shortening) as a within subjects factor. Bonferroni post-hoc comparisons were made between contraction modes where appropriate. T-tests for paired samples were performed to compare maximal voluntary torque with triplet torque evoked at rest, both expressed relative to their respective isometric torque, for lengthening and shortening conditions. There was one subject who had difficulties reaching a high maximal voluntary torque in the shortening contractions when a triplet was superimposed. Therefore, it was difficult to determine this subject's MTGC and voluntary activation level and these data were not used for analysis.

The Kolomogorov-Smirnov test was used to check for normality of the metabolite data of Experiment 2. As the data was not normally distributed, non-parametric Kruskall Wallis test was applied followed by a Mann-Whitney U test for post-hoc comparisons. For this analysis the PCr/Cr ratios of the three fibre populations (type I, IIA and IIAX fibres) were grouped and tested for significant differences compared to rest and between the three contractions modes (lengthening, isometric and shortening).

To investigate the activation of the individual fibre populations (type I, IIA and IIAX fibres) during lengthening, isometric and shortening contractions, cumulative distributions of the PCr/Cr ratios of individual fibre fragments were calculated for each fibre type, using intervals of 0.1. To determine significant differences between activation of the fibre populations, Kolmogorov-Smirnov two-sample tests were performed on the cumulative distributions. This test detects differences in both the location and the shape of the distributions (Siegel, 1956). The level of significance for all statistical analysis was set at $P<0.05$.

RESULTS

Experiment 1

Optimum knee angle

On average the highest relative torques were obtained at 60º knee flexion angle (97.8%). However, there were no significant differences with 70º (97.5%) and 80º (92.4%) knee flexion angle. The mean ± SD optimum angle for lengthening, isometric and shortening contractions were not significantly different, 66 ± 4, 64 ± 7 and 63 ± 7° knee flexion angle, respectively.

Maximal voluntary torque

Mean voluntary torque during lengthening (270 ± 55 Nm) was significantly higher than mean torque during shortening contractions (199 ± 47 Nm, $P<0.05$), but not significantly different from mean isometric torque (252 ± 47 Nm)(Figure 2A). Mean isometric torque was significantly higher than mean torque during shortening ($P<0.05$).

Maximal torque generating capacity (MTGC)

Mean MTGC for lengthening contractions (342 ± 68 Nm) was significantly higher than for isometric (273 ± 54 Nm, P<0.05) and shortening contractions (213 ± 53 Nm, P<0.05)(Figure 2A). In addition, mean isometric MTGC was significantly higher than shortening MTGC (P<0.05).

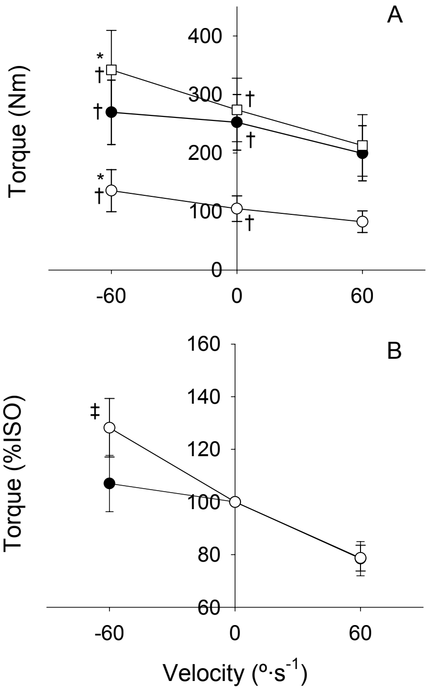

Figure 2. Mean ± SD A) Maximal voluntary torque (●), triplet torque (○) and estimated maximal torque generating capacity (□) during lengthening ($-60°·s^{-1}$), isometric ($0°·s^{-1}$) and shortening ($60°·s^{-1}$) contractions. *Indicates significant difference (P<0.05) compared to isometric or †shortening contractions. B) Maximal voluntary torque (●) and triplet torque (○) expressed relative to their respective isometric torque. ‡Indicates significant difference from relative maximal voluntary torque (P<0.05).

Triplet torque

Mean torque of the triplet evoked in resting muscle was significantly different between all modes of contraction, respectively 136 ± 36, 105 ± 22 and 82 ± 18 Nm for lengthening, isometric and shortening modes (P<0.05)(Figure 2A). When triplet torque and maximal voluntary torque for lengthening and shortening contractions were expressed relative to their respective isometric torque, it appeared that mean relative triplet torque (128 ± 11%) was significantly higher than mean relative maximal voluntary torque (106 ± 11%, $P<0.05$) for lengthening contractions, but not for shortening contractions (79 ± 5% and 78 ± 7%)(Figure 2B). This implies that voluntary activation was not complete during lengthening contractions.

Voluntary activation level

Mean voluntary activation level during lengthening contractions (79 ± 8%) was significantly lower than during isometric (93 ± 5%) and shortening contractions (92 ± 3%)($P<0.05$). There was no significant difference in voluntary activation level of isometric and shortening contractions (Figure 3).

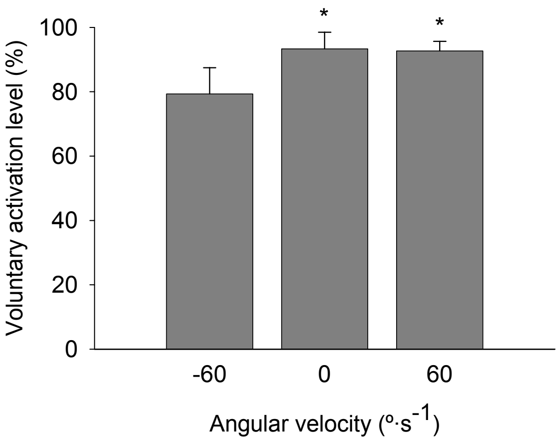

Figure 3. Mean ± SD voluntary activation level during lengthening (-60°·s^{-1}), isometric (0°·s^{-1}) and shortening (60°·s^{-1}) contractions. *Indicates significant difference from lengthening contractions ($P<0.05$).

Experiment 2

Maximal voluntary torque

Mean maximal voluntary torque data measured in the pre-sessions were not different from the data measured in Experiment 1: viz., 260 ± 58, 235 ± 34 and 208 ± 39 Nm, during lengthening, isometric and shortening contractions, respectively (One-way ANOVA). Mean maximal torque during the series of 10 lengthening, isometric and shortening contractions was 252 ± 61, 240 ± 20 and 213 ± 36 Nm, respectively.

Voluntary activation level

The mean activation level during the series of lengthening (76 ± 12%), isometric (90 ± 9%) and shortening (91 ± 10%) contractions were not different from the voluntary activation level of the three modes of exercise obtained in Experiment 1 (One-way ANOVA).

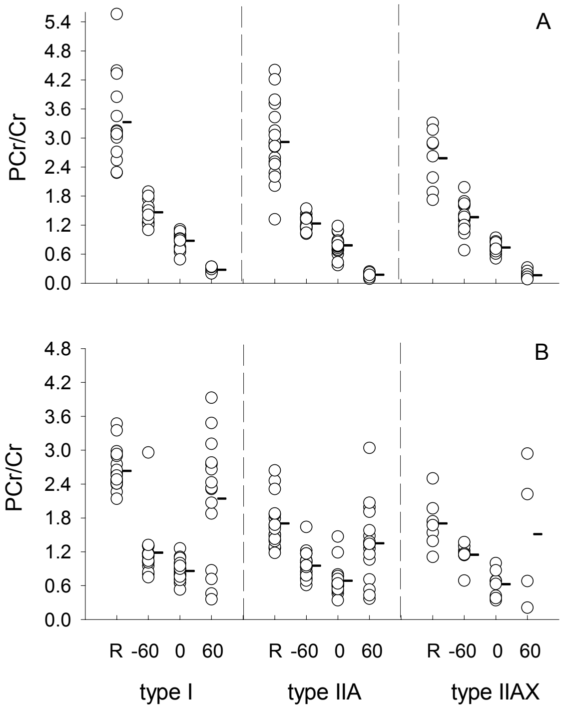

Figure 4. Example of PCr/Cr ratios of two different subjects (A, B) at rest (R) and after lengthening ($-60^{o}\cdot s^{-1}$), isometric ($0^{o}\cdot s^{-1}$) and shortening ($60^{o}\cdot s^{-1}$) contractions in type I, IIA and IIAX fibres. Mean value for the group of fibres is shown by –.

PCr/Cr ratio

A total of 720 single fibre fragments (293 type I, 289, type IIA and 138 type IIAX) were characterized and analysed for PCr and Cr content. Figure 4A shows an example of the PCr/Cr ratios of the different fibre fragments of one subject at rest and after exercise. The mean PCr/Cr ratio at rest for the type I fibres (2.5 ± 0.6, n = 94) was significantly different from the resting values of type IIA (2.0 ± 0.7, n = 95) and IIAX fibres (2.0 ± 0.7, n = 43)($P<0.05$). There were no significant

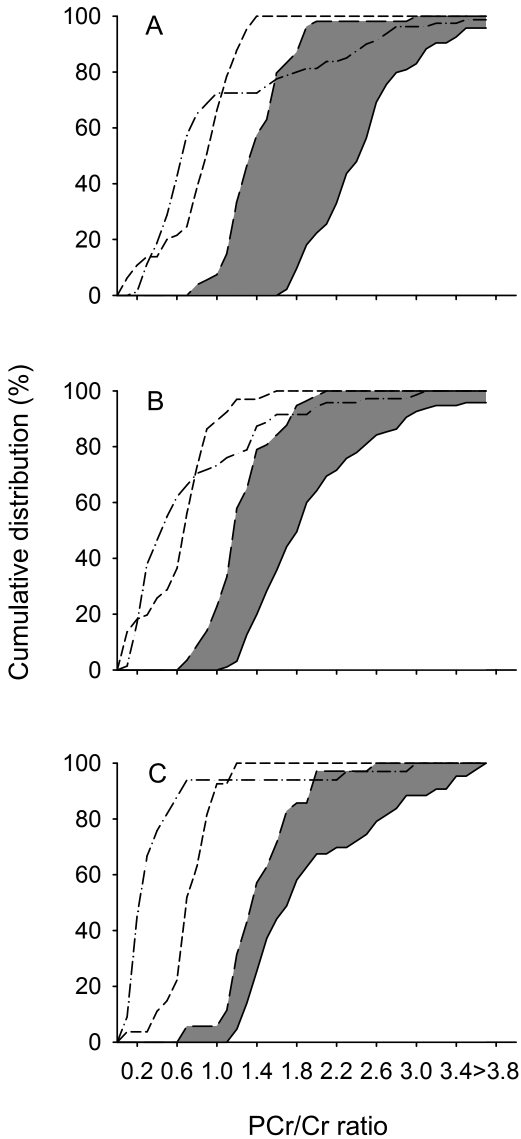

Figure 5. Cumulative distribution of single fibre PCr/Cr ratios in type I (A), IIA (B) and IIAX (C) fibres at rest (——) and after series of 10 lengthening (— —), isometric (----) and shortening (— · —) contractions. At rest, curves of all fibre types were significantly different ($P<0.05$). After each contraction mode the curves of all fibre types were significantly different from rest ($P<0.05$) and significantly different between contractions modes ($P<0.05$). The shaded areas in Figure A, B and C show that there is a less marked change in the PCr/Cr ratio after lengthening contractions for type IIAX fibres compared to type I fibres.

differences at rest between type IIA and IIAX fibres. After the series of lengthening, isometric and shortening contractions the mean PCr/Cr ratios for the grouped fibres were 1.3 ± 0.2 (n = 146), 0.7 ± 0.3 (n = 158) and 0.8 ± 0.6 (n = 184), respectively. These were all significantly different from resting values ($P<0.05$) and between contraction modes ($P<0.05$).

Cumulative distributions of the PCr/Cr ratio of the individual fibre populations are shown in Figure 5. Kolmogorov-Smirnov statistics showed that, at rest, the curves for type I, IIA and IIAX fibres were significantly different ($P<0.05$). For each fibre type, the distribution after either contraction mode was significantly different from rest ($P<0.05$). In addition, curves for lengthening, isometric and shortening contractions were significantly different for each fibre type ($P<0.05$). For all fibre types, there was an almost parallel shift in the curves for lengthening and isometric contractions, with a preservation of the shape of the curves at rest. However, the curves of type I and IIA fibres after shortening contractions show a collapse at PCr/Cr ratios of ~1.0. This is caused by the results of two subjects (for example, see Figure 4B) who show a relatively large variation in the PCr/Cr ratio after shortening exercise compared with the other three subjects (for example, see Figure 4A).

DISCUSSION

The present study has two main findings. First, the voluntary activation level during lengthening contractions was significantly lower than during isometric and shortening contractions, without a difference between the latter two. Second, PCr/Cr ratios of single characterized fibre fragments obtained from needle biopsies were decreased in all fibre types following 10 brief contractions and there was no evidence for a selective activation of type II fibres during lengthening contractions. In fact quite the opposite, associated with lower voluntary activation (~76% MTGC) there was a less marked change in the PCr/Cr ratio in the higher hierarchy IIAX fibres as might be normally expected from the size principle (see Figure 5).

Neural inhibition

In the present study, voluntary torque during maximal lengthening contractions was not higher than isometric torque. This is in contrast to what could be expected from force-velocity relationships of isolated muscle fibres (Edman et al., 1978) and whole muscle preparations (Katz, 1939) in which torque during lengthening has been shown to be higher than during isometric contractions. On the other hand, our results are comparable with studies on humans, which also did not find an increased torque during maximal lengthening contractions (Babault et al., 2001; Dudley et al., 1990; Westing et al., 1988; Westing et al., 1990). A reduced neural drive during lengthening contractions compared with isometric and shortening contractions is suggested to limit maximal voluntary torques (Webber and Kriellaars, 1997; Westing et al., 1990). Several results from the present study confirm the notion of a limited neural activation during lengthening contractions. First, direct evidence comes from the voluntary activation level calculated from superimposed stimulation. Our data show that the voluntary activation level for lengthening contractions was lower than for isometric and shortening contractions (Figure 3). Second, while voluntary torque during lengthening was not different from isometric torque, triplet torque during lengthening was 30% higher than the isometric triplet torque (Figure 2A). Third, the triplet torque during lengthening contractions was 20% higher compared to maximal voluntary torque when they were expressed relative to isometric torque (Figure 2B), while there was no difference for shortening contractions. Fourth, and as a consequence estimated MTGC was 27% and 62% higher in lengthening contractions compared to isometric and shortening contractions, respectively (Figure 2A, $P<0.05$).

A comparison of the data from experiment 1 can be made with a study from Babault et al. (2001). They also found lower activation levels during lengthening (88.3%) compared to isometric contractions (95.2%). Although in contrast to the present findings and previous studies (Amiridis et al., 1996; Westing et al., 1990) they also found a significant difference in activation levels during isometric compared to shortening contractions (89.7%).

Activation pattern

A reduced neural drive during lengthening contractions, as demonstrated by the present study, has been attributed to either preferential recruitment of type II motor units (that is, with a concomitant inhibition of type I fibre recruitment) or lower activation levels of all activated fibres (Enoka, 1996; Webber and Kriellaars, 1997). In the present study, PCr/Cr ratios of single characterized fibre fragments were used to investigate the activation pattern of different fibre populations during maximal lengthening, isometric and shortening contractions. We have calculated cumulative distributions and a significant shift in the curve of a fibre type after exercise was interpreted as an activation of that fibre type (Figure 5). The data show that all fibre types were active during attempted maximal lengthening, isometric and shortening contractions as, in each fibre type, there was a significant shift in the distribution after each contraction mode. In addition, for all fibre types there were significant differences between curves of the three modes of contractions. This was also seen in the mean decline of the PCr/Cr ratio of grouped fibre populations, which was significantly smaller for lengthening (37%, $P<0.05$) than for isometric (66%, $P<0.05$) and concentric contractions (67%, $P<0.05$). For lengthening contractions it might have been expected that the cumulative distribution would show a smaller shift in the curve since in an earlier study on maximally stimulated rat muscle we have shown that the PCr/Cr ratio after 10 maximally stimulated lengthening contractions was smaller compared to shortening contractions (Beltman et al., 2004b). Moreover and in addition, the mechanical data of the present study already showed that subjects had lower voluntary activation levels during lengthening contractions.

The fact that isometric and shortening distributions also differed was less expected because voluntary activation levels were not different. Furthermore in a study on rat muscle we showed that there was no difference in the PCr/Cr ratio after isometric and shortening contractions at similar relative contraction velocities of ~1/3 of optimum velocity (Beltman et al., 2004b). It should however be noted that the cumulative distribution curves for shortening contractions are different in shape when compared to lengthening and isometric contractions. This is attributed to the data of two subjects, who showed an unusually large variation in the PCr/Cr ratio after shortening exercise (for example, see Figure 4B). As the Kolmogorov-Smirnov two-sample test, assesses the difference in both position and shape, it is most likely that the significant difference between isometric and shortening curves is caused by the change in shape. The difference in shape is mostly attributable to a lower number of fibres with PCr/Cr ratios around 1.0.

It has been suggested that during submaximal lengthening exercise, there would be a selective activation of type II fibres and deactivation of type I fibres (Howell et al., 1995; Linnamo et al., 2003; Nardone et al., 1989). However, the present study could not find evidence for this concept in attempted maximal exercise. In contrast, as can be seen in Figure 5, the area of the grey areas for the three fibre populations decreases from type I to IIA to IIAX fibres. Thus, if there would be a difference in activation between fibre types our data seem to suggest that there is less activation of type IIAX compared to type I fibres. This observation is entirely consistent with a hierarchy of fibre type recruitment from I to IIA to IIAX. Moreover, it could even be speculated that there was a de-recruitment of type I fibres during shortening contractions as the shift in the curves after this mode of contraction was smaller than in the type IIA and IIAX fibres.

Conclusion

The present study investigated the voluntary activation level during lengthening, isometric and shortening contractions. In addition, the activation pattern of muscle fibre types was studied using the PCr/Cr ratio of single characterized fibre fragments as a measure of fibre activation. It was demonstrated that the voluntary activation level during lengthening contractions was significantly lower than during isometric and shortening contractions, while the degree of voluntary activation was not different between isometric and shortening contractions. The PCr/Cr ratio of single fibres showed that there was no evidence for selective recruitment of type II fibres with concomitant de-recruitment of type I fibres during lengthening contractions. In contrast, the data seem to suggest that, during lengthening contractions, all fibre populations were recruited albeit at a lower rate. Furthermore the PCr/Cr depletion pattern indicated a hierarchical pattern of motor unit recruitment.

ACKNOWLEDGEMENTS

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CHAPTER 7

Summarising discussion/ samenvatting en discussie

METABOLICALLY ASSESSED FIBRE RECRUITMENT

PCr/Cr ratio: a new marker for fibre activation

The first aim of the present thesis was to develop a method to investigate recruitment patterns of different fibre populations during only a few isometric or dynamic knee extension contractions. For this purpose a newly developed technique was adopted that consists of measuring PCr and Cr in single fibre fragments of which the fibre type was also characterized. Using this technique, Karatzaferi et al. (1999) and Sant'Ana Pereira et al. (1995b) have shown that energy metabolites (ATP, IMP, PCr and Cr) can be sensitively and reproducibly measured in single fibre fragments. Subsequently, this method has been used to study changes in metabolites during short-term whole body cycling exercise and during recovery (Karatzaferi et al., 2001a; Karatzaferi et al., 2001b; Sant'Ana Pereira et al., 1996).

In the present thesis this method was applied, using the quadriceps muscle as a model (Chapter 2), to establish whether the decrease in the PCr/Cr ratio of single fibre fragments could be used as a marker for fibre activation. The first question was how long (or brief) the duration of the knee extensor exercise had to be to produce a significant decline in the PCr/Cr ratio that would allow fibre activation to be detected (Chapter 3). Needle biopsies were obtained after 4, 7 and/or 10 maximal voluntary contractions of 1 s duration (1 s rest). Single fibre data showed that after only 7 contractions the PCr/Cr ratio of (almost) all fibre fragments was below the predetermined threshold (5^{th} percentile of resting values) and it was concluded that this exercise duration was sufficient to identify activated fibres.

As the resynthesis of PCr is very fast, the half-time is ~30 s (Harris et al., 1976; Kushmerick and Meyer, 1985; Sahlin et al., 1979), it is of great importance that needle biopsies are obtained and frozen in liquid nitrogen as quickly as possible after relaxation. To guarantee the sensitivity of the method the maximal sampling time, which is the time from the end of exercise to the freezing of the sample, was set beforehand as maximally 10 s. If this time was exceeded either no sample was obtained or the sample was discarded and hence not analysed (Chapter 3, 4 and 6). Another choice in the set-up of the exercise protocol, which is related to the fast resynthesis of PCr, is the rest period between contractions. This was kept as short as possible (1 s) in all studies in which needle biopsies were obtained (Chapter 3, 4 and 6).

Even though this technique is somewhat laborious, the results of the present thesis show that this method allows information to be obtained on recruitment patterns, which cannot be attained with other available methods. Though single motor unit EMG and the glycogen depletion method can certainly be used to assess recruitment of motor units or muscle fibres the method presented in this thesis has numerous new possibilities. Most of these have been addressed in the different chapters (Chapter 1, 3, 4 and 6) but an overview is presented here.

First, using the mATPase stability-based histochemistry (Brooke and Kaiser, 1970; Sant'Ana Pereira et al., 1995b), the fibre fragments isolated from needle biopsies can be characterized (type I, IIA and IIAX)(Chapter 3, 4 and 6). Clearly, in the glycogen depletion method the same technique can also be used to determine the fibre type (e.g. Vøllestad et al., 1984; Vøllestad and Blom, 1985; Vøllestad et al., 1992) but, in single motor unit EMG, findings on recruitment patterns are often based on the size of the identified motor units (Freund, 1983). Furthermore, with the present method numerous fibres of the same type can be assessed, although it remains unknown whether these are from the same or from different motor units.

Second, this new method allows exercise lasting for only a very few seconds to be studied (Chapter 3, 4 and 6) as the immediate source of ATP resynthesis is by the PCr kinase system (McComas, 1996; Spriet, 1995). The method of single motor unit EMG can of course study a single contraction, but the glycogen depletion method requires an exercise duration of at least ~10 min to detect a decline in the PAS staining intensity of the fibres in the cross-sectioned muscle sample. During 10 min of exercise there may be a sequential pattern of recruitment and, while at the end of exercise it can be concluded which fibres had been activated during the 10 min, the initial pattern of recruitment cannot be derived.

Third, besides isometric contractions (Chapter 3 and 4), the method presented in this thesis allows recruitment patterns to be assessed during dynamic contractions (Chapter 6). Due to technical problems this is very difficult using single motor unit EMG. The main problem is the electrode position, which can change as result of the contraction and thus it is very difficult to follow motor unit action potentials from the start to the end of the contraction (McComas, 1996).

Fourth, another new possibility of the PCr/Cr ratio as a measure of fibre activation is that even during strong, (near) maximal contractions activation of individual fibres can be assessed (Chapter 3, 4 and 6). Using the glycogen depletion method, recruitment patterns for supramaximal exercise (cycling at >100% VO_2max) have also been assessed (Gollnick et al., 1973b; Vøllestad et al., 1992), but this is very difficult using needle or wire electrodes. Again, technical problems limit the possibilities of the latter method. As many motor units are firing in the area of the electrode, it becomes almost impossible to identify single motor unit action potentials (McComas, 1996).

And last, recruitment patterns assessed using single motor EMG have mostly studied relatively small muscles such as the first dorsal interosseus (Carpentier et al., 2001; De Luca et al., 1982; Desmedt and Godaux, 1977b; Howell et al., 1995; Milner-Brown et al., 1973), tibialis anterior (Desmedt and Godaux, 1977a; Feiereisen et al., 1997; Hannerz, 1974), triceps surae (Nardone and Schieppati, 1988; Nardone et al., 1989) and toe extensors (Grimby and Hannerz, 1977). The present thesis has demonstrated that, with the new technique, it is possible to study activation patterns of a large muscle (vastus lateralis muscle). In glycogen depletion studies muscle biopsies have also been obtained from this muscle, but mostly whole body movement has been investigated, such as cycling. Though the glycogen depletion method would also allow dynamic knee extension contractions to be studied, isometric contractions are more difficult. Kernell et al. (1995) have shown that, due to differences in glycogen degradation rates of different fibre types, there was no significant decline in the glycogen content in type I fibres after 6 s of maximally activated isometric contractions in rat muscle. In this context it is worth noting that glycogen degradation in the different fibre types also depends on other available substrates (Essén, 1978; Kernell et al., 1995). Moreover, it has been shown that glycogen depletion occurs even in non-exercised (suspended) rat muscles as a result of adrenaline released during exercise of the other legs (McDermott et al., 1987). Thus, since there are multiple factors contributing to the differences in glycogen depletion in the different fibre types, it has been suggested that glycogen depletion as a measure for fibre activation should be used with care (Kernell et al., 1995).

Single muscle fibre recruitment during brief exercise

As our new method was shown to be reproducible (Karatzaferi et al., 1999) and useful for the detection of fibre activation (Chapter 3), it was applied to actually study recruitment of three different fibre types (type I, IIA and IIAX) during isometric and dynamic contractions. With increasing intensity (Chapter 4) it was found that fibres were recruited orderly in a hierarchical pattern:

$$I \rightarrow IIA \rightarrow IIAX$$

This does not seem to be a new, surprising finding as this is according the *size principle* (Henneman et al., 1965) and has been confirmed using the glycogen depletion method as a measure for fibre activation (Gollnick et al., 1973a; Gollnick et al., 1974a; Vøllestad et al., 1984; Vøllestad and Blom, 1985). However, it must be realised that the unique value of the present result is that it was assessed after only 7 contractions while the glycogen depletion data was after a minimum of 10 min of exercise.

While the present method does not allow making clear statements on the contribution of fibre recruitment or rate coding at different intensities (see "Limitations and recommendations") some notable suggestions could be made. First, it was hypothesised that a further decrease in the PCr/Cr ratio of type I fibres at the highest intensity (87% of maximal voluntary contraction torque) was a result of an increased firing rate of already active motor units. This was a surprising finding as it might have been expected that at 72% of maximal voluntary contraction torque all type I fibres would have been fully activated. Second, from the significant, but relatively small (compared to the other intensities) decrease in the PCr/Cr ratio, it was hypothesised that some type IIA fibres were already active at 39% of maximal voluntary contraction torque. These type IIA fibres were not at all expected to be active at this intensity as type I fibres alone should be able to produce the required torque at an intensity less than ~50% of maximal voluntary torque (Sargeant and Jones, 1995). The shift in the distribution of the PCr/Cr ratio of type IIA fibres at this relatively low intensity was suggested to be an indication of submaximal activation of this fibre population. A similar suggestion was made by Vøllestad et al. (1984). However, with their protocol they exercised for 20 min, which made it impossible to determine the sequence of recruitment in time. Consequently, with that study, based on glycogen depletion data, it is impossible to conclude that type IIA fibres were recruited from the start of exercise.

An early submaximal activation of type IIA fibres would be functional in submaximal exercise as the physiological demand is distributed over more fibre types, thus preventing selective fatigue (Ivy et al., 1987). In addition, the strategy of recruiting type IIA fibres during low level (endurance) exercise would prevent atrophy of these fibres when muscles are mainly used in low to moderate exercise (Ivy et al., 1987).

The results of the experiment after submaximal and maximal contractions (Chapter 4) showed that the present technique is very valuable for assessing recruitment patterns during isometric contractions at different intensities. In the last part of the thesis the focus was on energy cost, activation level and recruitment during dynamic contractions. The main interest was on lengthening contractions, as there is a lack of knowledge on recruitment patterns during this mode of exercise. It is relatively well established that in voluntary lengthening contractions lower torques are produced than can be expected from the force-velocity relationship of skeletal muscles. Though this is attributed to a limitation of the neural drive by many investigators (Webber and Kriellaars, 1997; Westing et al., 1991), the underlying mechanism for this inhibition has still not been described. In the present thesis (Chapter 6) a reduced neural drive during lengthening contractions was confirmed using superimposed electrical stimulation on maximal voluntary effort. Besides a lower voluntary activation level, some more indirect measures provided evidence for inhibition of the neural drive such as a greater maximal torque generating capacity and torque of triplet on relaxed muscle in lengthening compared with isometric contractions. In addition, PCr/Cr ratios measured from fibre fragments obtained after 10 lengthening contractions exhibited a smaller decline than after the same number of isometric and shortening contractions. However, in Chapter 5 it was shown that the energy cost for mechanical output was also lower for lengthening contractions when muscles were *maximally* activated. Rat gastrocnemius muscles were used as a model in this experiment in which was attempted to simulate human movement. The advantage of such a model is that muscles can be maximally activated by electrical stimulation via the innervating nerve. The lower energy flux during lengthening contractions was attributed to intrinsic muscle properties presumable within the cross-bridges that are forcibly detached and thus require less ATP (Flitney and Hirst, 1978). So, it must be realised that the smaller decline in the ratio after 10 voluntary lengthening contractions, compared to isometric and shortening contractions, in Chapter 6 could be a combination of lower energy fluxes on the one hand and a lower voluntary activation on the other hand.

Using the PCr/Cr ratio of single characterized fibres, the present study gave more insight into the mechanisms underlying a lower neural drive during lengthening contractions (Chapter 6). For submaximal lengthening contractions (up to ~40% maximal voluntary contraction torque), it has been suggested that there is a preferential recruitment of fast type II fibres with a concomitant de-recruitment of slow type I fibres (Howell et al., 1995; Nardone et al., 1989). Nardone et al. (1989) argue that, for lengthening contractions, motor units need to be activated which have appropriate relaxation times to control lowering a load. They suggested that this would require activation of fast motor units having fast relaxation rates, as slow motor units will cause a too slow descent. Moreover, it might be suggested that type I fibres consume energy during lengthening contractions but they will not be able to contribute to force production.

The results of the present thesis (Chapter 6) could not support the suggestion of selective type II fibre activation, as there was a significant shift in the cumulative frequency distribution to lower PCr/Cr ratios for *all* fibre populations after lengthening contractions. Taking into account that the decline in the PCr/Cr ratio was a combined effect of lower energy cost and lower voluntary activation, it was suggested that during lengthening contractions all fibre populations were recruited, albeit at a lower level. It was also speculated that, in lengthening contractions, type IIAX fibres were activated to a lower extent compared to type I fibres. Selective activation of fast muscle fibre types could also not be demonstrated by others (Kossev and Christova, 1998; Søgaard et al., 1996) using single motor unit EMG measured at low intensity exercise (10-25% of maximal voluntary torque). According to Bawa and Jones (1999), the reversal of motor unit recruitment as suggested by Nardone et al (1989) and Howell et al. (1995), is most likely a result of “hesitations” during the lengthening contractions causing fast units to become recruited at lower thresholds. Thus, Bawa and Jones (1999) suggested that the phenomenon of selective recruitment does not represent a special recruitment strategy during these types of contractions.

Limitations and recommendations

Some methodological issues need to be addressed which may limit the practical application of the method used. The dissection of single fibre fragments, characterizing the fibres and measuring PCr and Cr content, requires a specialised set-up and a considerable experience. Furthermore, as single fibre fragments need to be analysed both for fibre type and metabolites, the procedure is rather time consuming. This is also one of the reasons why a relatively small number of subjects participated in the experiments of Chapter 3, 4 and 6. Moreover, obtaining muscle samples within 10 s after relaxation was not always successful. As a consequence not all subjects had data for all conditions. It might be suggested that a small number of subjects and missing data would make it difficult to extrapolate the results to a larger population. However, overall the data give a consistent presentation of the effect of activation on the PCr/Cr ratio of different fibre types.

The force produced by a single muscle is the combined result of the number of recruited motor units and firing rates of those motor units. A limitation of the present method is that it does not allow a clear distinction between these two force-regulating mechanisms because a reduction in the PCr/Cr ratio is a result of both recruitment and rate coding. A complicating factor is further that the cross-bridge turnover rates are different between fibre types and hence ATP turnover rates. So, a different reduction in the ratio does not necessarily indicate a different activation level. Yet, by combination of results of different exercises some suggestions could be made with respect to rate coding (Chapter 3 and 6).

In Chapter 6 the activation of different fibre populations was assessed during maximal lengthening, isometric and shortening contractions. It has been suggested that during dynamic contractions the recruitment threshold is lower compared with isometric contractions (Linnamo et al., 2003; Theeuwen et al., 1994). This would mean that certain fibre types are recruited at lower intensities during dynamic contractions. However, as muscle samples were obtained after maximal efforts for each exercise mode, it was not possible to draw any conclusions on activation of each fibre type at different intensities of dynamic exercise.

Selective recruitment of fast motor units has been suggested in fast, low force lengthening contractions. With the present method a preferential activation of fast fibres could not be demonstrated in maximal attempted lengthening contractions at a relatively low velocity. However, it cannot be ruled out that different results can be obtained at different intensities and movement velocities.

Despite its limitations, the method can be a useful tool for studying activation in different modes of exercise. Moreover, adaptations in activation strategies as a result of training, disuse and neural/neuromuscular disorders can be studied to obtain a better understanding of the plasticity of the human neuromuscular system.

METABOLE BEPALING VAN VEZELREKRUTERING

PCr/Cr ratio: een nieuwe marker voor vezelactivatie

Het eerste doel van dit proefschrift was om een methode te ontwikkelen waarmee rekruteringspatronen van verschillende vezelpopulaties onderzocht kunnen worden tijdens slechts een paar isometrische of dynamische knie extensie contracties. Hiervoor werd een pas ontwikkelde techniek gebruikt waarbij de phosphocreatine (PCr) en creatine (Cr) inhoud wordt gemeten in stukjes van enkele vezels waarvan vooraf het vezeltype bepaald is. Karatzaferi et al (1999) en Sant'Ana Pereira et al. (1995b) hebben aangetoond dat met deze techniek metabolieten (adenosine triphosphate (ATP), inosine monophosphate (IMP), PCr en Cr) gevoelig en herhaalbaar gemeten kunnen worden in stukjes van enkele vezels. In latere studies is deze methode ook gebruikt om veranderingen in metabolieten te bestuderen tijdens en na kortdurende maximale fietsinspanning (Karatzaferi et al., 2001a; Karatzaferi et al., 2001b; Sant'Ana Pereira et al., 1996).

In dit proefschrift is deze techniek gebruikt, met de m. quadriceps als een model (Hoofdstuk 2), om te bepalen of een afname in de PCr/Cr ratio van afzonderlijke vezels gebruikt kon worden als een marker voor vezelactivatie. De eerste vraag was hoe lang (of hoe kort) de inspanning (knie extensies uitgevoerd op een dynamometer) moest zijn om een aanzienlijke afname in de PCr/Cr ratio te krijgen die voldoende was om de geactiveerde vezels waar te nemen (Hoofdstuk 3). Hiervoor werden spierbiopten afgenomen na 4, 7 en/of 10 maximaal vrijwillige contracties van 1 s, met 1 s rust tussen de contracties. De resultaten van de afzonderlijke vezels lieten zien dat na slechts 7 contracties de PCr/Cr ratio van (bijna) alle vezels onder de, van tevoren vastgestelde drempel (5^e percentiel van rustwaardes) lag. De conclusie was dat deze inspanningsduur voldoende was om geactiveerde vezels waar te nemen.

Aangezien de resynthese van PCr erg snel is, na ~30 s is de voorraad al voor de helft hersteld (Harris et al., 1976; Kushmerick and Meyer, 1985; Sahlin et al., 1979), is het erg belangrijk dat de spierbiopten zo snel mogelijk na de inspanning genomen worden en worden ingevroren in vloeibare stikstof. Om de gevoeligheid van de methode te waarborgen is van tevoren vastgesteld dat de maximale biopt-afname-tijd (tijdsduur vanaf relaxatie na inspanning tot het invriezen van het biopt) niet langer mocht zijn dan 10 s. Als het langer duurde werd er geen biopt genomen of werd het verkregen biopt niet geanalyseerd (Hoofdstuk 3, 4 en 6). Een andere keuze in de opzet van het inspanningsprotocol, ook gerelateerd aan de snelle resynthese van PCr, is de rustperiode tussen de contracties. Deze was dan ook zo kort mogelijk (1 s) in alle studies waar spierbiopten werden afgenomen (Hoofdstuk 3, 4 en 6).

Ondanks dat deze techniek tamelijk arbeidsintensief is, laten de resultaten van dit proefschrift zien dat met deze methode informatie over rekruteringspatronen verkregen kan worden welke niet verkregen kan worden met andere beschikbare methodes. Natuurlijk kunnen motor unit EMG en de glycogeen-depletie methode gebruikt worden om rekruteringspatronen van motor units of spiervezels te bestuderen. Echter, de methode beschreven in dit proefschrift geeft een aantal nieuwe mogelijkheden. De meeste van deze mogelijkheden zijn in de afzonderlijke hoofdstukken aan bod geweest (Hoofdstuk 1, 3, 4 en 6) maar hieronder volgt een overzicht.

Als eerste, door gebruik te maken van histochemische kleuringen die gebaseerd zijn op de stabiliteit van mATPase bij verschillende pH's (Brooke and Kaiser, 1970; Sant'Ana Pereira et al., 1995b), kan van ieder stukje vezel dat uit het spierbiopt is gehaald het vezeltype (type I, IIA en IIAX) worden bepaald (Hoofdstuk 3, 4 en 6). Het is duidelijk dat in de glycogeen-depletie methode dezelfde techniek gebruikt kan worden om vezeltypes te bepalen (bijv.Vøllestad et al., 1984; Vøllestad and Blom, 1985; Vøllestad et al., 1992). Echter, bij motor unit EMG zijn de bevindingen over rekruteringspatronen vaak gebaseerd op de grootte van de gemeten motor units (Freund, 1983). Bovendien kan met de huidige methode een groot aantal vezels van hetzelfde type worden bestudeerd, hoewel het onduidelijk blijft of deze tot dezelfde of andere motor units behoren.

Ten tweede, met deze nieuwe methode kan een inspanning van slechts een paar seconden worden bestudeerd (Hoofdstuk 3, 4 en 6) aangezien onmiddellijk resynthese van ATP wordt verzorgd door het creatine kinase systeem (McComas, 1996; Spriet, 1995). Met motor unit EMG kan ook één enkele contractie bestudeerd worden, maar voor de glycogeen-depletie methode is een inspanningsduur van minimaal ~10 minuten vereist om een waarneembare daling in de intensiteit van de PAS (Periodic Acid Schiff) kleuring te krijgen in de dwarsdoorsneden van de spierbiopten. Tijdens deze 10 minuten inspanning kan er een opeenvolgende inschakeling zijn van vezels. Aan het eind van de inspanning kan dan wel geconcludeerd worden welke vezels actief zijn geweest maar niet wat het initiële rekruteringspatroon was.

Ten derde, naast het bestuderen van isometrische contracties (Hoofdstuk 3 en 4) kunnen, met de methode beschreven in dit proefschrift, ook rekruteringspatronen worden bestudeerd tijdens dynamische contracties (Hoofdstuk 6). Door technische problemen is dit erg moeilijk met behulp van motor unit EMG. Het grootste probleem is de elektrode positie, deze kan veranderen als gevolg van de contractie. Hierdoor is het erg moeilijk om bepaalde motor unit actiepotentialen te volgen van het begin tot het eind van de contractie (McComas, 1996).

Ten vierde, een andere nieuwe mogelijkheid van de PCr/Cr ratio als maat voor vezelactivatie is dat de activatie van afzonderlijke vezels zelfs kan worden bepaald tijdens krachtige, (bijna) maximale contracties (Hoofdstuk 3, 4 en 6). Met de glycogeen-depletie methode zijn rekruteringspatronen ook bestudeerd tijdens supra-maximale inspanning (fietsen op >100% VO2max) (Gollnick et al., 1973b; Vøllestad et al., 1992), maar dit is erg moeilijk met behulp van naald- of draadelektrodes. Weer zijn het technische problemen die een beperking vormen voor de laatst genoemde methode. Aangezien er veel motor units in de buurt van de elektrode vuren, is het bijna onmogelijk om de afzonderlijke motor unit actiepotentialen waar te nemen (McComas, 1996).

En als laatste, met behulp van motor unit EMG zijn rekruteringspatronen voornamelijk bestudeerd in relatief kleine spieren zoals de m. eerste interosseus dorsales (Carpentier et al., 2001; De Luca et al., 1982; Desmedt and Godaux, 1977b; Howell et al., 1995; Milner-Brown et al., 1973), de m. tibialis anterior (Desmedt and Godaux, 1977a; Feiereisen et al., 1997; Hannerz, 1974), de m. triceps surae (Nardone and Schieppati, 1988; Nardone et al., 1989) of de m. extensor digitorum brevis (Grimby and Hannerz, 1977). In dit proefschrift is aangetoond dat het met de nieuwe techniek mogelijk is om activatiepatronen te bestuderen van een grote spier (m. vastus lateralis). In studies waar gebruik gemaakt is van de glycogeen-depletie methode zijn ook spierbiopten van deze spier gebruikt maar deze studies hebben voornamelijk algehele lichamelijke inspanning bestudeerd, zoals fietsen. Ofschoon het met de glycogeen-depletie methode ook mogelijk is om dynamische knie extensie contracties te bestuderen is dit moeilijker voor isometrische contracties. Kernell et al. (1995) hebben aangetoond dat, door de verschillen in de snelheid van glycogeen afbraak in verschillende vezeltypen, er geen significante afname was in de glycogeen inhoud in type I vezels na 6 s maximaal geactiveerde isometrische contracties in rattenspieren. In deze context moet ook genoemd worden dat de afbraak van glycogeen in de verschillende vezeltypen ook afhankelijk is van andere beschikbare substraten (Essén, 1978; Kernell et al., 1995). Bovendien is aangetoond dat glycogeen depletie zelfs optreedt in niet geactiveerde rattenspier doordat adrenaline vrijkomt tijdens de inspanning van de andere spieren (McDermott et al., 1987). Dus, aangezien er meerdere factoren zijn die bijdragen aan de variatie in glycogeen depletie in verschillende vezeltypen, wordt gesuggereerd dat de glycogeen methode als maat voor vezelactivatie met de nodige voorzichtigheid gebruikt moet worden (Kernell et al., 1995).

Rekrutering van afzonderlijke vezels tijdens kortdurende inspanning

Aangetoond is dat de methode herhaalbaar (Karatzaferi et al., 1999) en geschikt is om vezelactivatie waar te nemen (Hoofdstuk 3). Vervolgens is de methode toegepast om daadwerkelijk rekrutering te bestuderen van drie verschillende vezeltypen (type I, IIA en IIAX) tijdens isometrische en dynamische contracties. Met toenemende intensiteit (Hoofdstuk 4) is gevonden dat de vezels geordend gerekruteerd werden in een hiërarchisch patroon:

$$I \rightarrow IIA \rightarrow IIAX$$

Dit lijkt geen nieuw, verrassend resultaat aangezien dit volgens het size principle is (Henneman et al., 1965) en ook bevestigd is met de glycogeen-depletie methode als maat voor vezelactivatie (Gollnick et al., 1973a; Gollnick et al., 1974a; Vøllestad et al., 1984; Vøllestad and Blom, 1985). Wel moet echter gerealiseerd worden dat het unieke van deze resultaten ligt in het feit dat het gemeten is na slechts 7 contracties terwijl de resultaten van de glycogeen-depletie methode gevonden waren na een inspanning van minimaal 10 minuten.

Ondanks dat het met de huidige methode niet mogelijk is om duidelijke uitspraken te doen over de bijdrage van vezelrekrutering of rate coding bij verschillende intensiteiten (zie “Beperkingen en aanbevelingen”) konden er toch een aantal opmerkelijke suggesties gedaan worden. Als eerste werd verondersteld dat de verdere afname in de PCr/Cr ratio van de type I vezels bij de hoogste intensiteit (87% van maximaal vrijwillige contractie kracht) het gevolg was van een toename in vuurfrequentie van de al geactiveerde motor units. Dit was een verrassend resultaat aangezien verwacht mocht worden dat bij 72% van de maximaal vrijwillige kracht alle type I vezels volledig geactiveerd zouden zijn. Ten tweede, doordat er een significante, maar relatief kleine (vergeleken met de andere intensiteiten) afname was in de PCr/Cr ratio van de type IIA vezels, werd verondersteld dat sommige type IIA vezels al actief waren bij 39% van de maximaal vrijwillige contractie kracht. Het was niet verwacht dat type IIA vezels al actief zouden zijn bij deze intensiteit aangezien de type I vezels alleen in staat zouden moeten zijn de gevraagde intensiteit van minder dan ~50% van maximale vrijwillige kracht te leveren (Sargeant and Jones, 1995). De verschuiving in de distributie van de PCr/Cr ratio van type IIA vezels bij deze, relatief lage, intensiteit lijkt te wijzen op een submaximale activatie van deze vezelpopulatie. Een vergelijkbare suggestie is gedaan door Vøllestad et al. (1984). Echter, hun inspanningsprotocol duurde 20 minuten, wat het onmogelijk maakt om de volgorde van rekrutering in de tijd vast te stellen. Als gevolg hiervan is het aan de hand van die studie, waarvan de resultaten zijn gebaseerd op glycogeen depletie, onmogelijk te concluderen dat type IIA vezels vanaf het begin van de inspanning gerekruteerd zijn geweest.

Een vroege, submaximale activatie van type IIA vezels kan functioneel zijn in submaximale inspanning aangezien de fysiologische belasting dan verdeeld is over meerdere vezeltypen waardoor selectieve vermoeidheid vermeden wordt (Ivy et al., 1987). Bovendien zou de strategie waarin type IIA vezels gerekruteerd worden bij lage intensiteit (uithoudingsvermogen) voorkomen dat er in deze vezels atrofie optreedt als de spieren voornamelijk gebruikt worden in inspanning met een lage tot matige intensiteit (Ivy et al., 1987).

De resultaten van het experiment na submaximale en maximale contracties (Hoofdstuk 4) lieten zien dat de huidige techniek erg waardevol is voor het bepalen van rekruteringspatronen tijdens isometrische contracties bij verschillende intensiteiten. Het laatste deel van dit proefschrift concentreerde zich op de energetische kosten, activatie niveau en rekrutering tijdens dynamische contracties. De nadruk lag op actieve verlenging aangezien er een gebrek is aan kennis over rekruteringspatronen tijdens dit soort contracties. Het is redelijk goed bekend dat tijdens maximaal vrijwillige actieve verlenging lagere momenten worden geleverd dan verwacht kan worden naar aanleiding van de kracht-snelheids relatie van skeletspieren. Ondanks dat dit door veel onderzoekers wordt toegeschreven aan een remming van de neurale aansturing (Webber and Kriellaars, 1997; Westing et al., 1991), zijn de onderliggende mechanismen voor deze remming nog steeds niet beschreven. In dit proefschrift (Hoofdstuk 6) is met behulp van elektrische stimulatie gegeven boven op maximaal vrijwillige contracties, bevestigd dat er een verminderde neurale aansturing is tijdens actieve verlenging. Naast een verminderde vrijwillige activatie waren er een aantal, meer indirecte, aanwijzingen voor een verminderde neurale aansturing. Tijdens verlenging werd er een hoger maximaal moment genererend vermogen gevonden, evenals een hoger moment veroorzaakt door een triplet op een ontspannen spier, beide vergeleken met isometrische contracties. Daarnaast was de daling in de PCr/Cr ratio gemeten in afzonderlijke vezels na 10 actieve verlengingen kleiner dan na hetzelfde aantal isometrische contracties en actieve verkortingen. Echter, in Hoofdstuk 5 was aangetoond dat de energetische kosten voor mechanische output lager was tijdens actieve verlenging van spieren die maximaal waren geactiveerd. De m. gastrocnemius van de rat werd in deze studie als model gebruikt waarbij zoveel mogelijk werd geprobeerd menselijk bewegen na te bootsen. Voordeel van zo'n model is dat spieren maximaal geactiveerd kunnen worden met behulp van elektrische stimulatie via de zenuw die de spier innerveert. De lagere energetische flux tijdens actieve verlenging werd toegeschreven aan intrinsieke spiereigenschappen, waarschijnlijk op het niveau van de cross-bridges. Tijdens actieve verlenging wordt een deel van deze cross-bridges mechanisch ontkoppeld door externe krachten op de spier wat minder ATP kost (Flitney and Hirst, 1978). Dus, het is belangrijk te realiseren dat de lagere afname in de ratio na 10 vrijwillige actieve verlengingen, vergeleken met isometrische contracties en actieve verkortingen, in Hoofdstuk 6 een gevolg is van zowel lagere energetische fluxen als een lagere vrijwillige activatie.

Door gebruik te maken van de PCr/Cr ratio van afzonderlijke, getypeerde vezels gaf deze studie meer inzicht in de mechanismen die ten grondslag liggen aan de lagere neurale aansturing tijdens actieve verlenging (Hoofdstuk 6). Er wordt wel gesuggereerd dat tijdens submaximale actieve verlenging (tot ~ 40% van de maximaal vrijwillige contractie kracht) type II vezels bij voorkeur geactiveerd worden en type I vezels tegelijkertijd gedeactiveerd (Howell et al., 1995; Nardone et al., 1989). Nardone et al. (1989) pleiten dat, tijdens actieve verlenging, motor units geactiveerd moeten worden die een geschikte relaxatie tijd hebben om een gewicht beheerst te kunnen laten zakken. Volgens hen zouden de snelle motor units actief moeten zijn omdat deze een snelle relaxatie hebben. Langzame motor units zouden een last te langzaam laten zakken. Bovendien zou gesuggereerd kunnen worden dat type I vezels energie gebruiken tijdens actieve verlenging terwijl ze niet kunnen bijdragen aan de kracht leverantie.

De resultaten van dit proefschrift (Hoofdstuk 6) kunnen het voorstel van selectieve activatie van type II vezels niet ondersteunen aangezien, na actieve verlenging, alle vezelpopulaties een significante verschuiving lieten zien van de cumulatieve frequentie distributie naar lagere PCr/Cr ratio's. Rekening houdend met het feit dat de afname in de PCr/Cr ratio een combinatie is van een lagere energetische belasting en een lager activatie niveau, is gesuggereerd dat alle vezelpopulaties gerekruteerd zijn geweest, zij het op een lager niveau. Er werd ook gespeculeerd dat tijdens actieve verlenging type IIAX vezels minder geactiveerd waren dan de type I vezels. Onderzoekers die gebruik maakten van motor unit EMG, gemeten tijdens submaximale (10-25% van de maximaal vrijwillige kracht) konden ook geen bewijs vinden voor selectieve activatie van snelle spiervezels (Kossev and Christova, 1998; Søgaard et al., 1996). Volgens Bawa & Jones (1999) wordt de omgekeerde motor unit rekrutering, zoals gesuggereerd door Nardone et al. (1989) en Howell et al. (1995), waarschijnlijk veroorzaakt door “haperingen” tijdens actieve verlengingen als gevolg van inschakeling van units met lagere rekruteringsdrempels. Dus, Bawa & Jones (1999) suggereren dat selectieve rekrutering niet echt een speciale rekruteringsstrategie is tijdens dit soort contracties.

Beperkingen en aanbevelingen

Een aantal methodologische punten dient hier behandeld te worden die de praktische toepassing van de methode kunnen beperken. Voor het isoleren van afzonderlijke vezelfragmenten, het bepalen van de vezeltypes en het meten van PCr en Cr inhoud is een gespecialiseerde opstelling en een aanzienlijke hoeveelheid ervaring nodig. Bovendien is de methode redelijk tijdrovend aangezien van de afzonderlijke vezelfragmenten zowel het vezeltype als de metabolieten gemeten moeten worden. Dit is ook een van de redenen dat er een relatief klein aantal proefpersonen mee deden in de experimenten van Hoofdstuk 3, 4 en 6. Daarnaast is het niet altijd gelukt een spierbiopt af te nemen binnen 10 s na het eind van de inspanning. Het gevolg hiervan is dat niet voor alle proefpersonen data beschikbaar waren voor alle condities. Gezien het kleine aantal proefpersonen en het ontbreken van data kan gesuggereerd worden dat het moeilijk is de resultaten te extrapoleren naar een grotere populatie. Desondanks, geven de data over het algemeen een consistent beeld van het effect van activatie op de PCr/Cr ratio van verschillende vezeltypen.

De kracht geleverd door een enkele spier is het resultaat van zowel het aantal gerekruteerde motor units als de vuurfrequentie van deze motor units. Een beperking van de huidige methode is dat geen duidelijk onderscheid gemaakt kan worden tussen deze twee krachtregulerende mechanismen. Dit komt omdat een afname in de PCr/Cr ratio het resultaat is van zowel rekrutering als rate coding. Een bemoeilijkende factor is verder dat de snelheid van de cross-bridge cyclus, en dus de snelheid waarmee ATP gebruikt wordt, verschillend is tussen vezeltypen. Dus een verschil in de afname van de PCr/Cr ratio hoeft niet te betekenen dat de vezels op een verschillend niveau geactiveerd zijn. Toch konden, door een combinatie van de resultaten van verschillende inspanningen, een aantal suggesties gedaan worden met betrekking tot rate coding (Hoofdstuk 3 en 6).

In Hoofdstuk 6 is de activatie van verschillende vezelpopulaties bepaald gedurende actieve verlenging, isometrische contractie of tijdens actieve verkorting. Er wordt gesuggereerd dat tijdens dynamische contracties de rekruteringsdrempels lager zijn vergeleken met isometrische contracties (Linnamo et al., 2003; Theeuwen et al., 1994). Dit zou betekenen dat bepaalde vezeltypen tijdens dynamische contracties op een lagere intensiteit geactiveerd worden. Echter, aangezien in de experimenten in Hoofdstuk 6 spierbiopten na maximale pogingen genomen zijn, was het niet mogelijk conclusies te trekken over de activatie van ieder vezeltype bij verschillende intensiteiten van dynamische inspanning.

Gesuggereerd wordt dat er een selectieve rekrutering van snelle motor units plaatsvindt, met name tijdens snelle, submaximale actieve verlengingen. Met de huidige experimenten kon deze voorkeur voor snelle vezels niet worden aangetoond in maximale contracties op een relatief lage snelheid. Het kan echter niet worden uitgesloten dat andere resultaten gevonden worden bij andere intensiteiten en bewegingssnelheden.

Ondanks de beperkingen kan de methode een bruikbaar meetinstrument zijn voor het bestuderen van vezelactivatie in verschillende soorten inspanning. Daarnaast kunnen aanpassingen in activatiestrategieën als gevolg van training, inactiviteit en neurale/neuromusculaire ziektes bestudeerd worden om een beter inzicht te krijgen in de plasticiteit van het neuromusculaire systeem.

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List of Publications

PAPERS IN INTERNATIONAL JOURNALS

Beltman, J. G. M., Sargeant, A. J., van Mechelen, W,. and de Haan, A. (2004). Lower activation level of human quadriceps muscle during lengthening contractions: no evidence for selective recruitment of type II fibers. *Revision submitted*.

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ABSTRACTS

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